

FIG. 1A

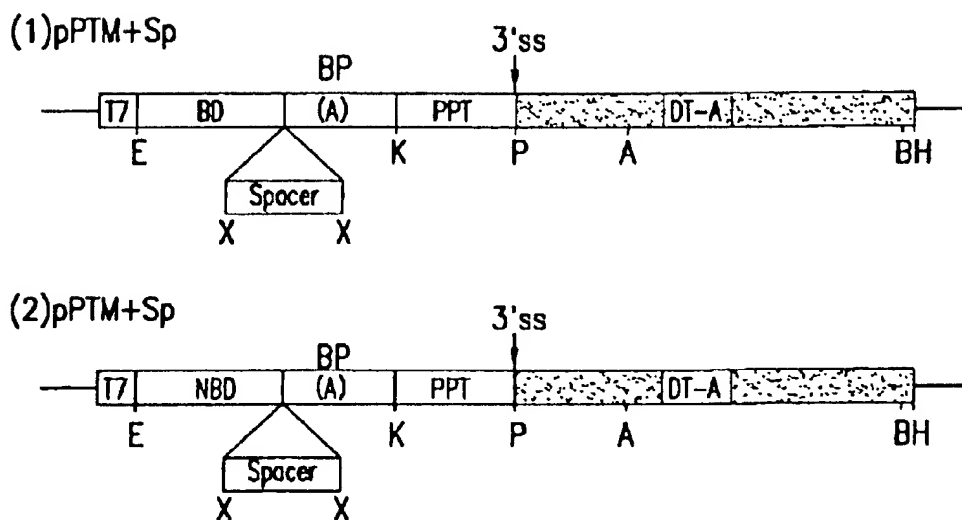


FIG.1B

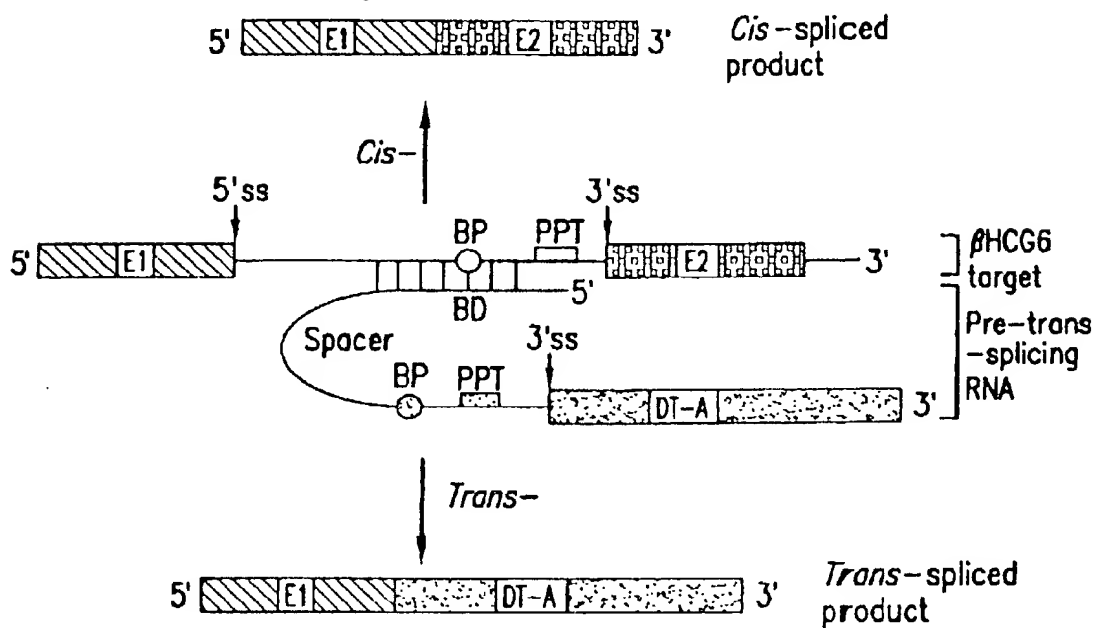


FIG.1C

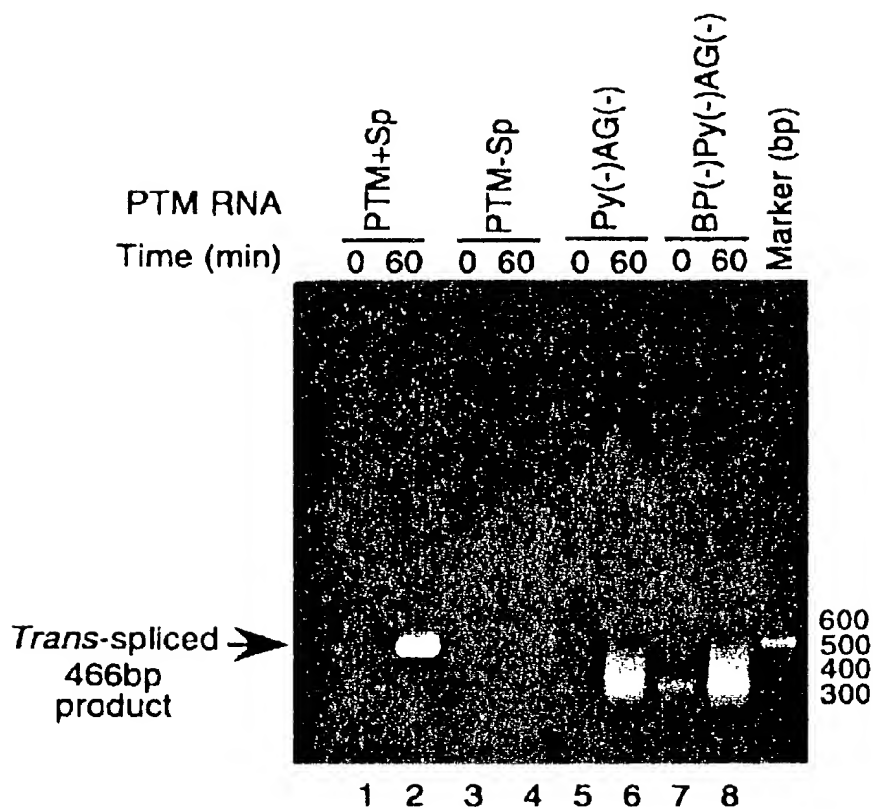


FIG.2A

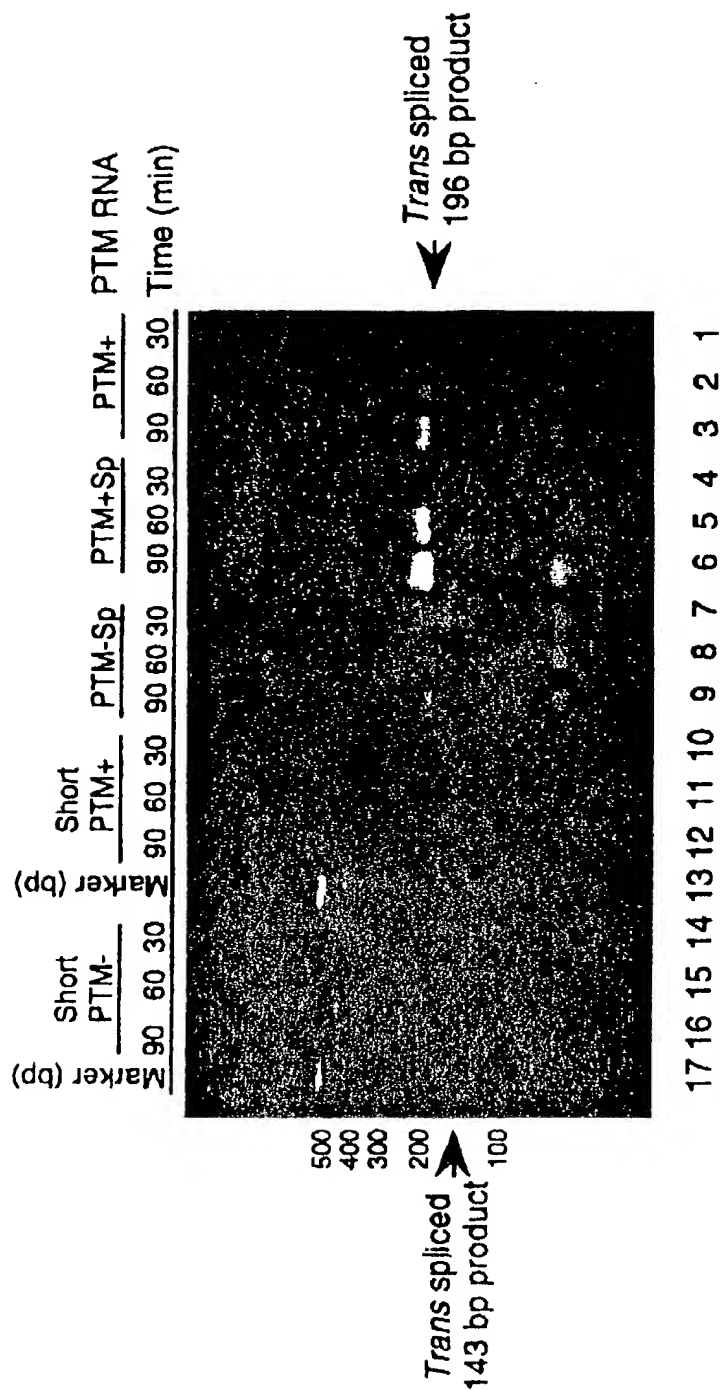


FIG.2B

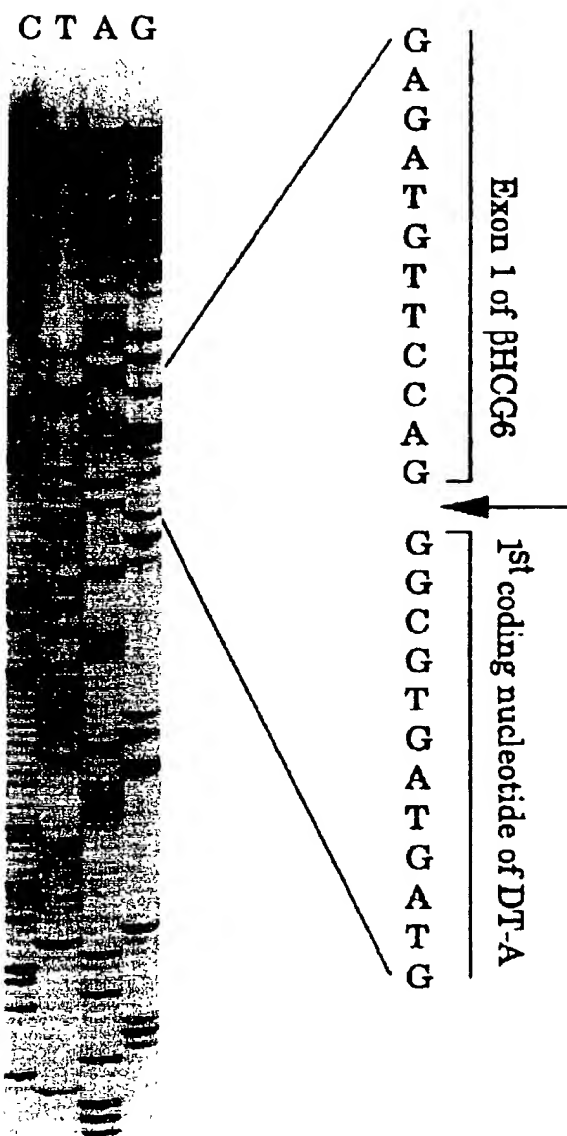
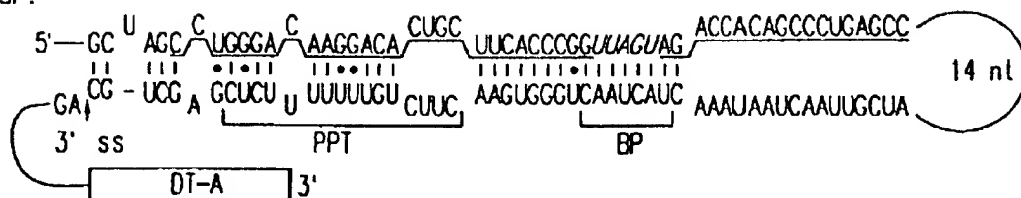
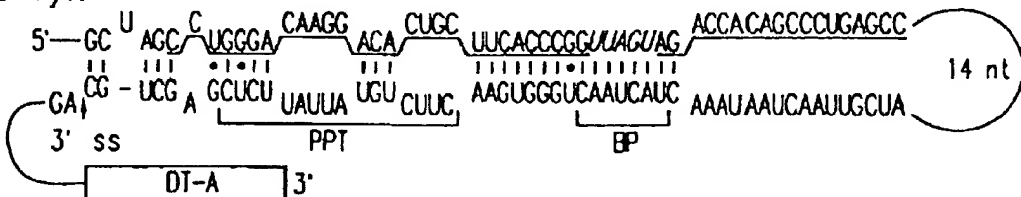


FIG.3

1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:

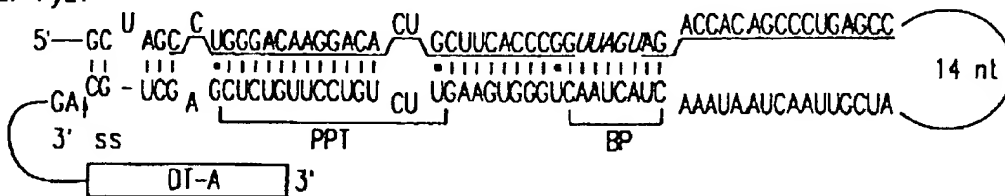


FIG.4A

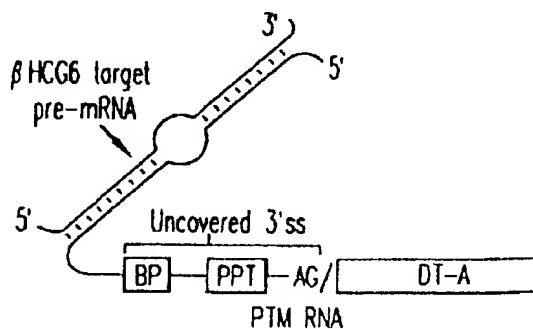


FIG.4B

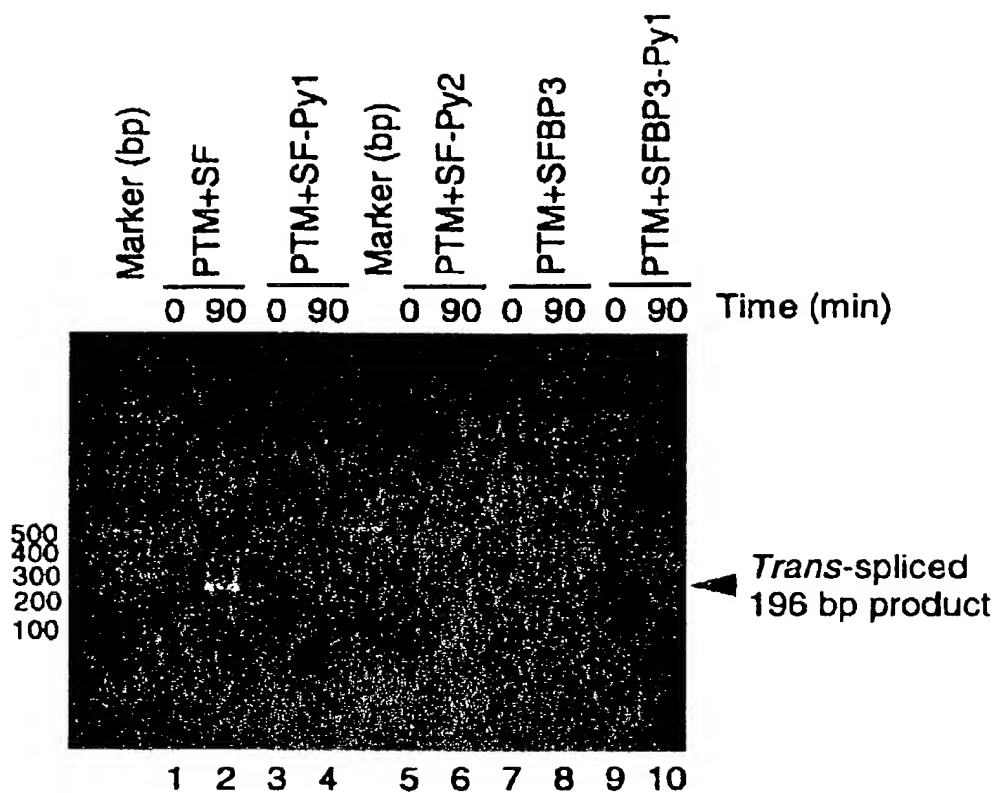


FIG.4C

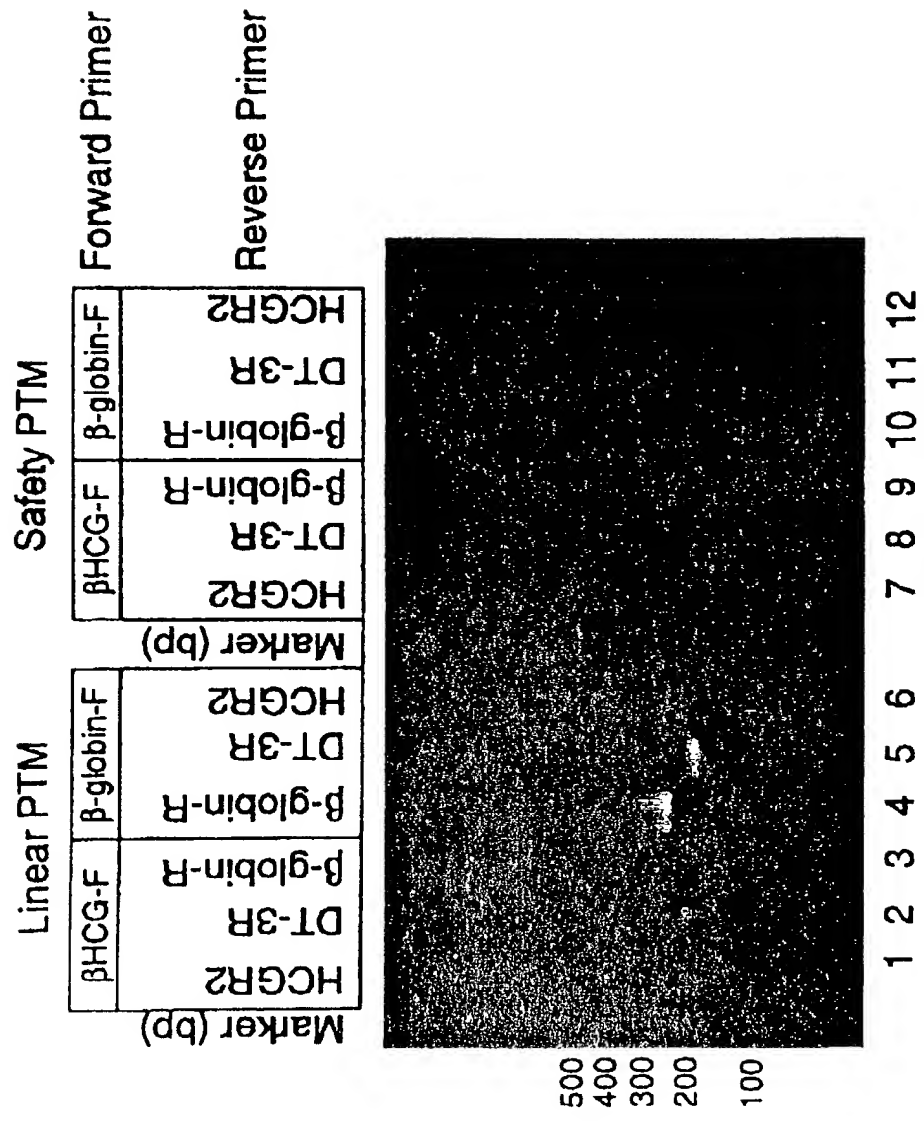


FIG.5

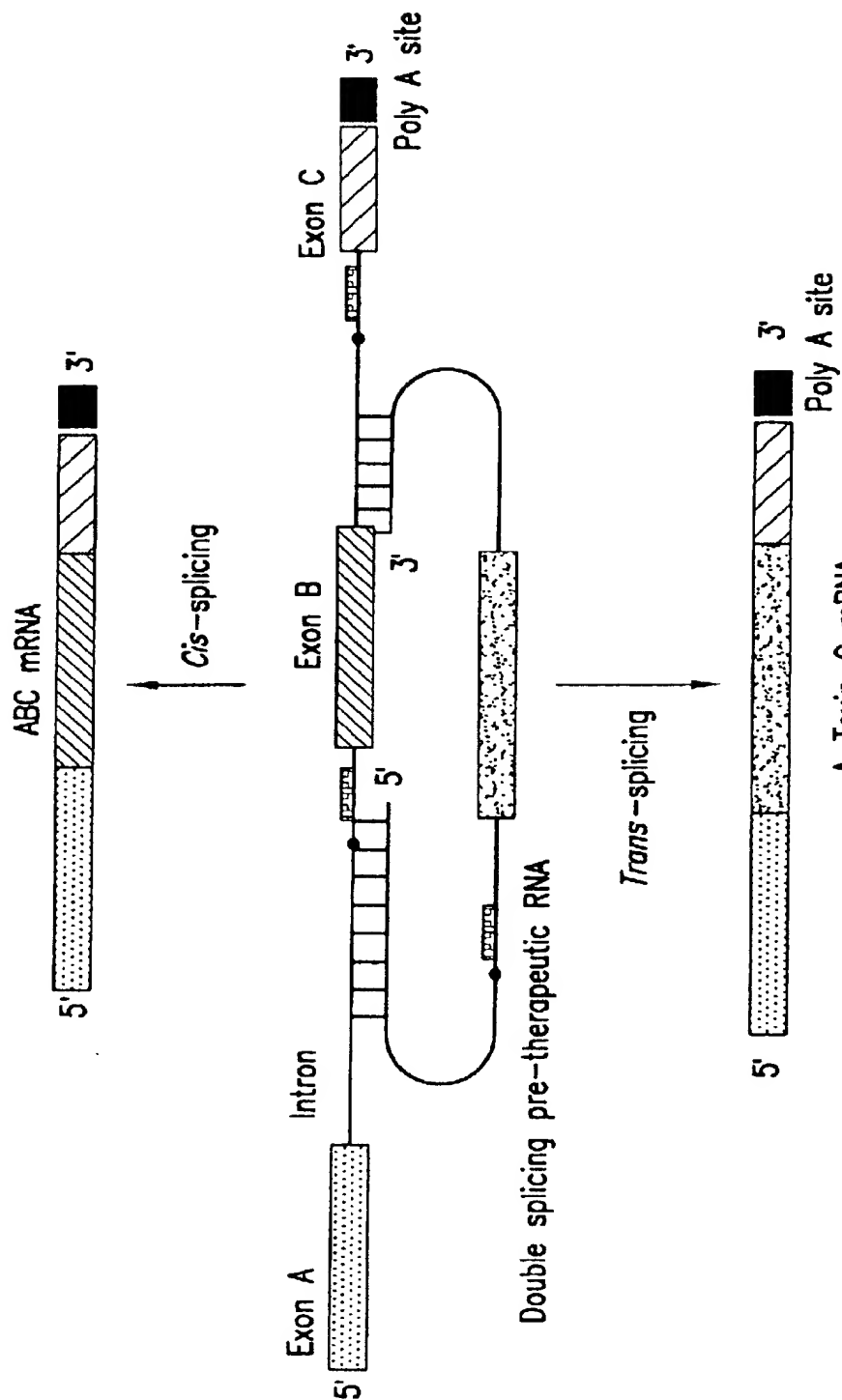


FIG.8A

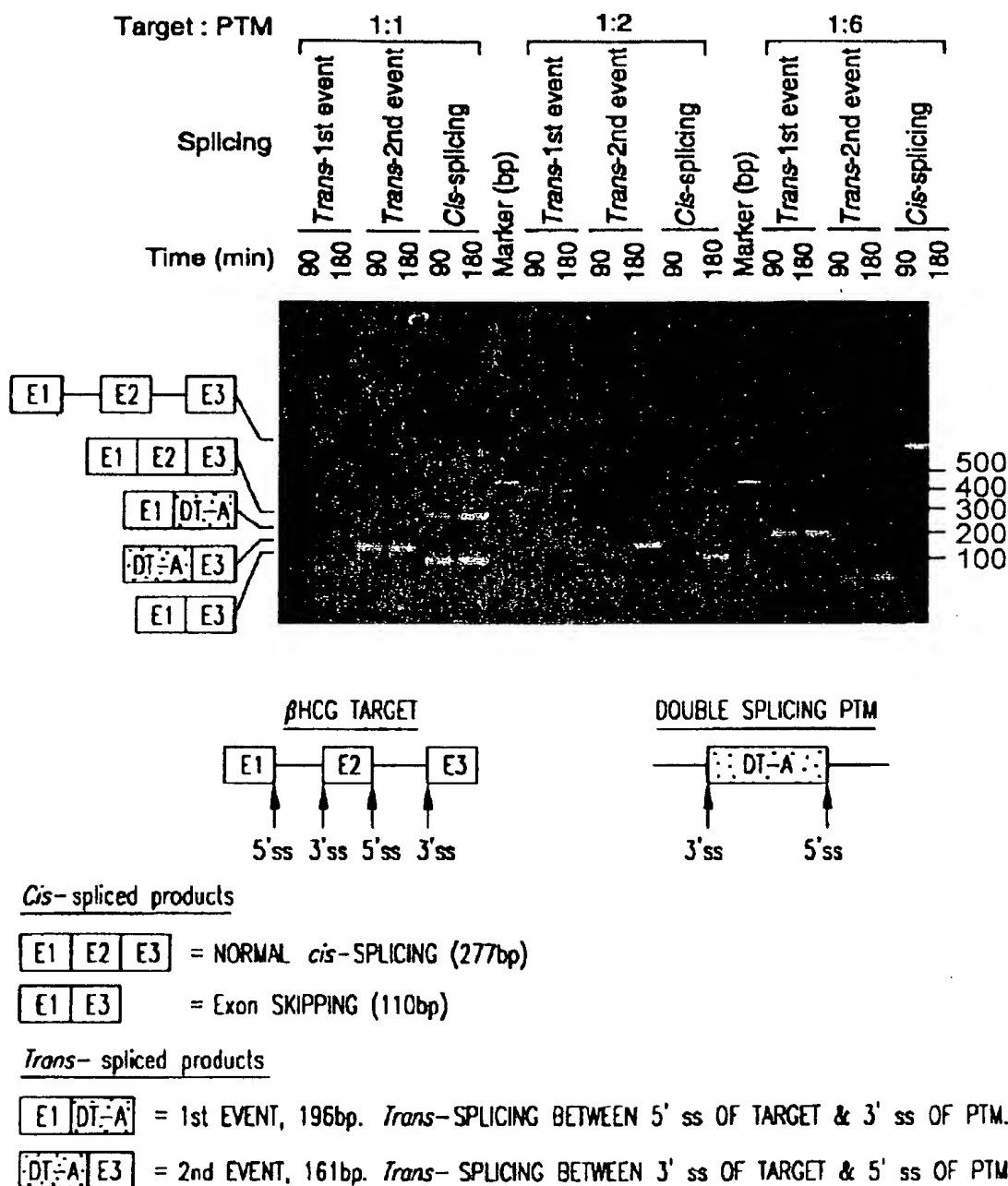


FIG.8B

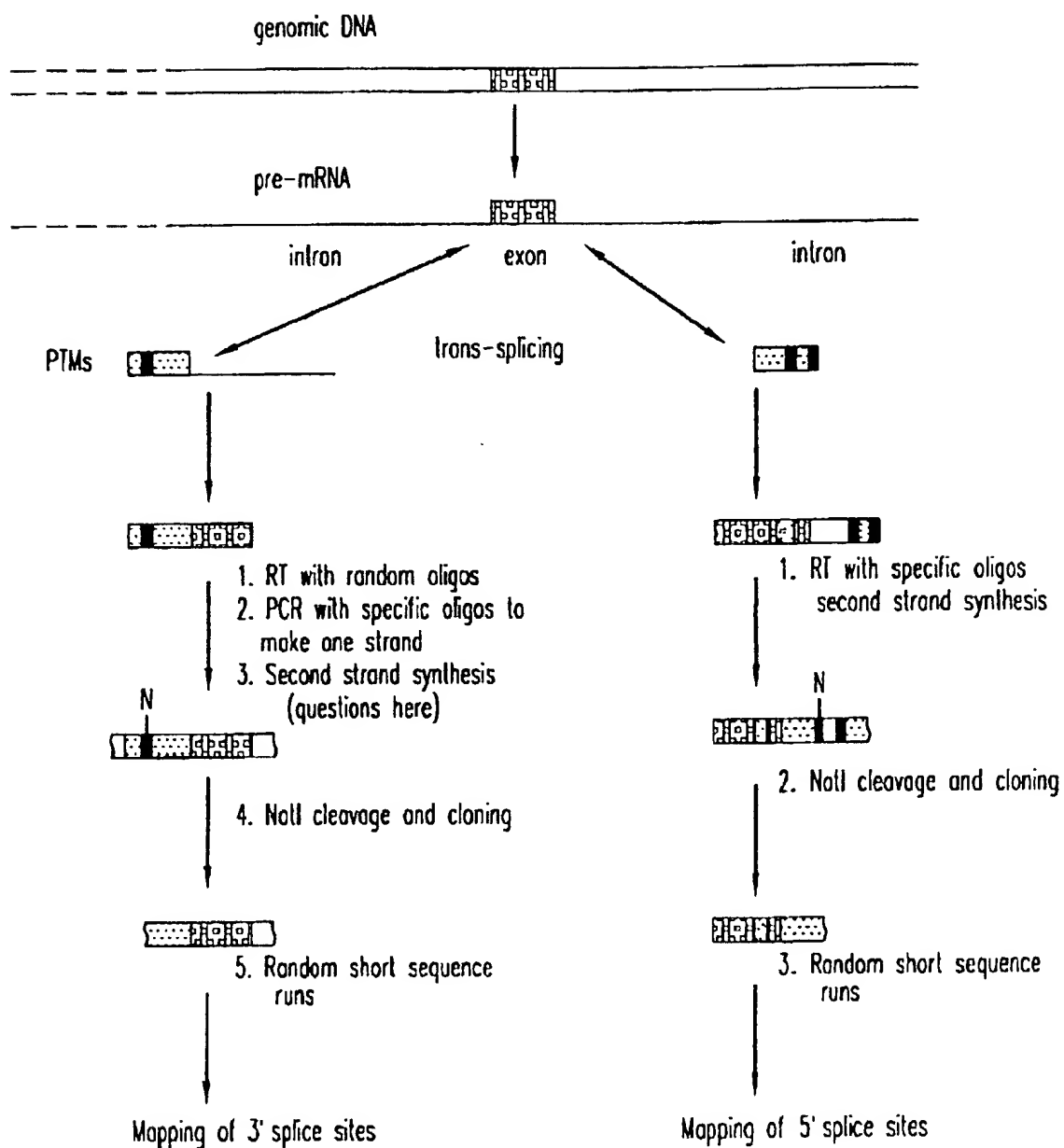


FIG.9

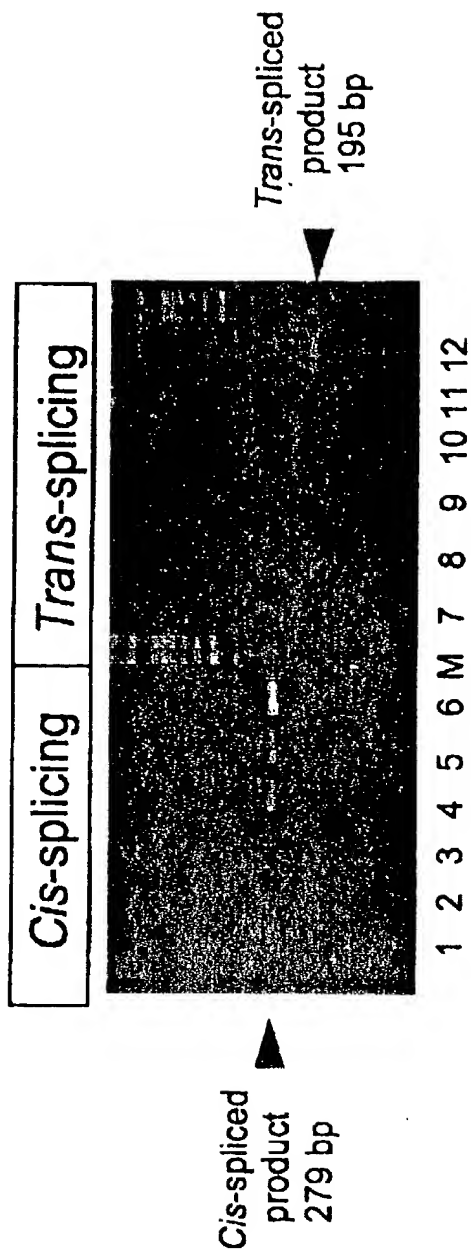


FIG.11A

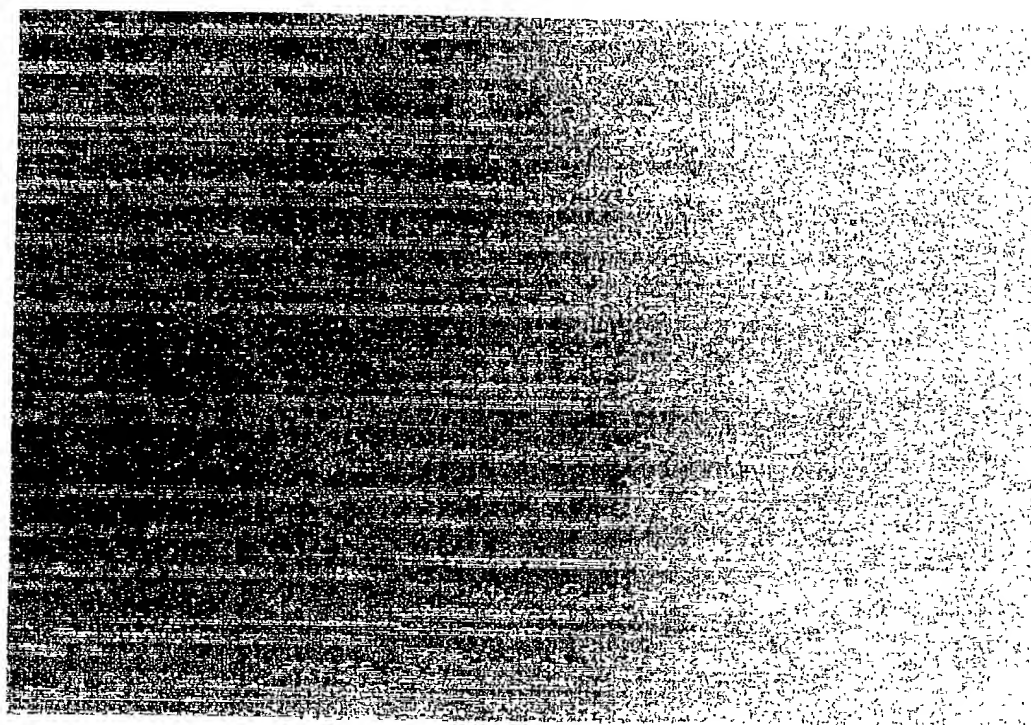


FIG.11B

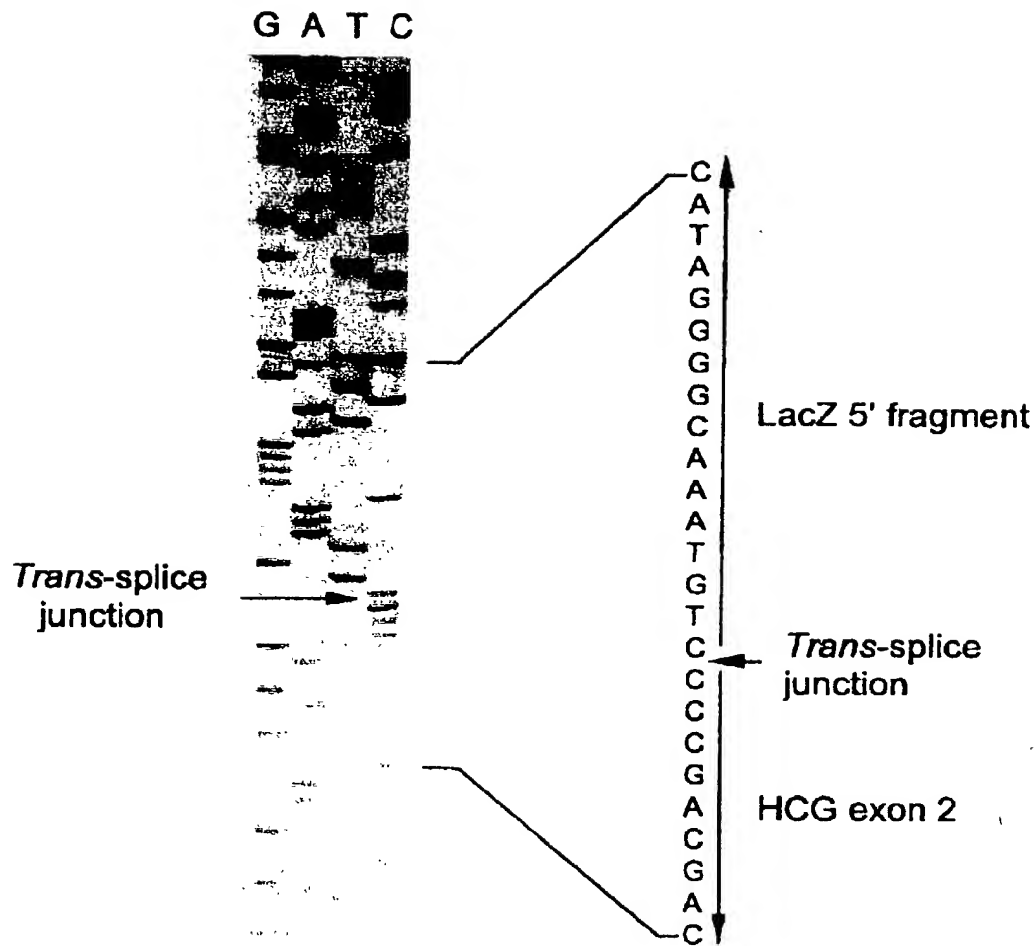


FIG.12A

1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPLICED PRODUCT (285 bp):

BioLac-TR1

GGCTTTGGCTACCTGGAGAGACGGCCCGCTGATCCTTTGGGAAATACGGCCACGGGATGGGTAAACAGTCTTG

GGCGTTTCGCTAAATACTGGCAGGGCTTTCGTGAGTATCCCGGTTTACAG/GGGCGCTTGGTCTAATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCGTGGTGGCTTACGGGGTGATTT

TGGCGATACGGCGAAGCATGGCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGGCGCATCCAG

Lac-TR2

2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

BioLac-TR1

GGCTTTGGCTACCTGGAGAGACGGCCCGCTGATCCTTTGGGAAATACGGCCACGGGATGGGTAAACAGTCTTG

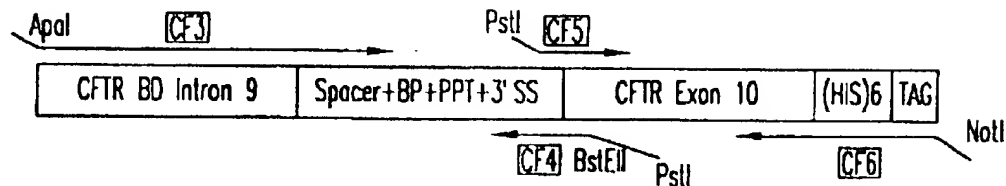
CGGTTTCGCTAAATACTGGCAGGGCTTTCGTGAGTATCCCGGTTTACAG/GGGCTGCTGCTGTTGCTGCTGCT

HCGR2

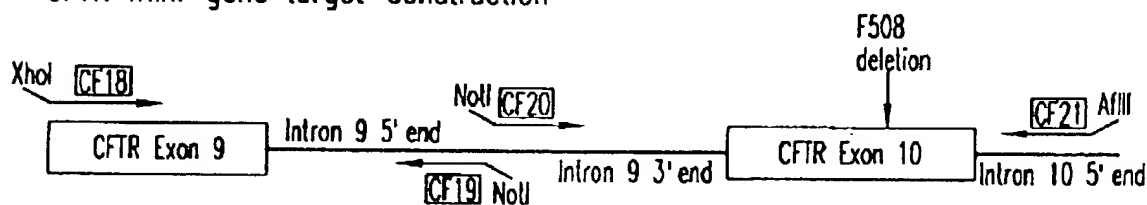
GAGCATGGGGGGACATGGGCATCCAAAGGAGCCACTTCGGCCACGGTGCCG

FIG. 12B

CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

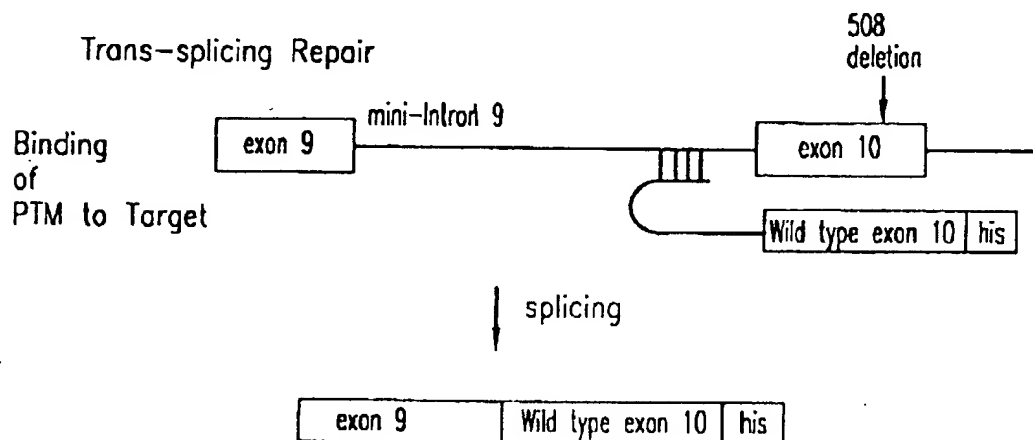


FIG.13

DNA sequence 500 b.p. GCTACCGTTTAA ... TGCCACTCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)

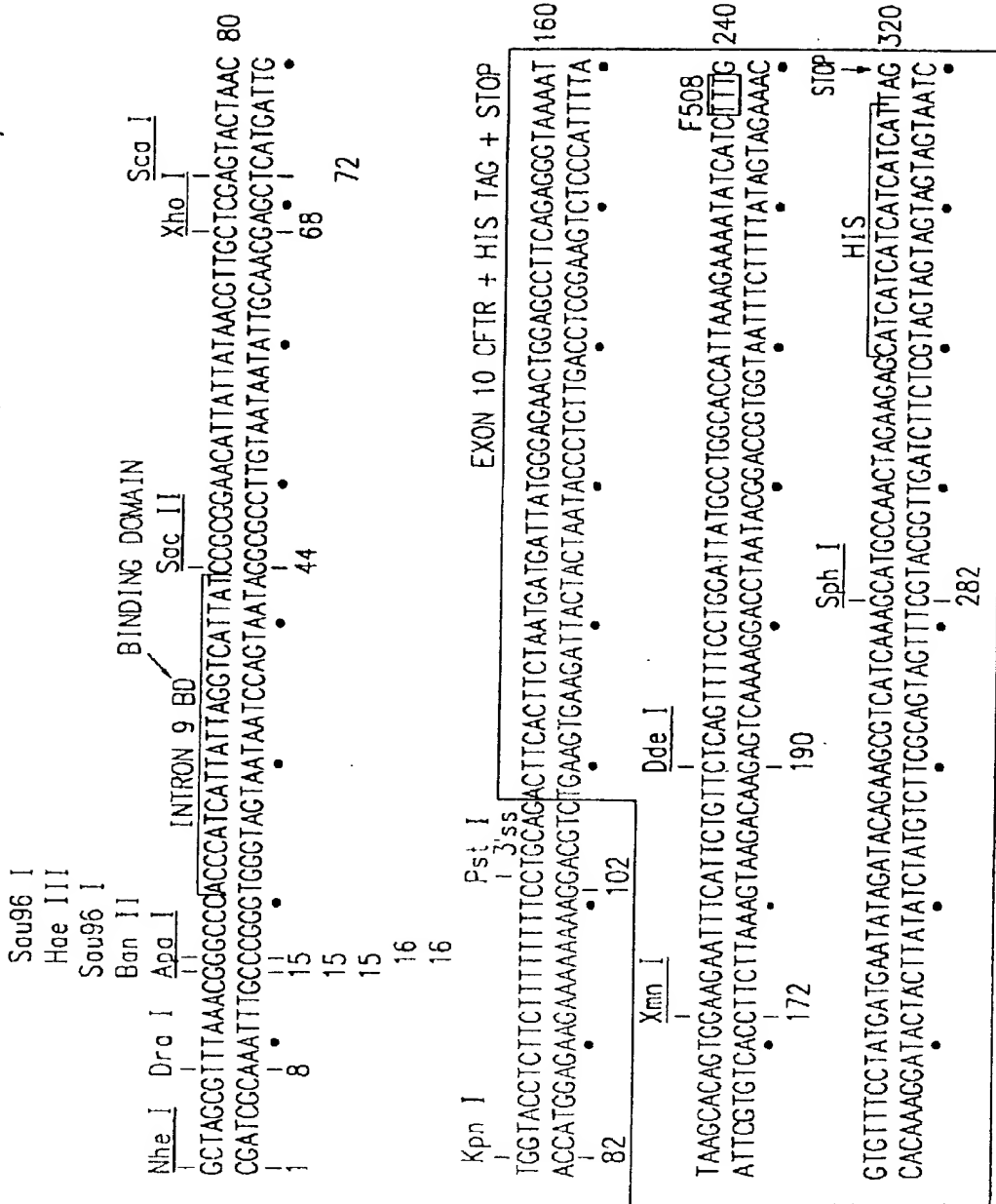
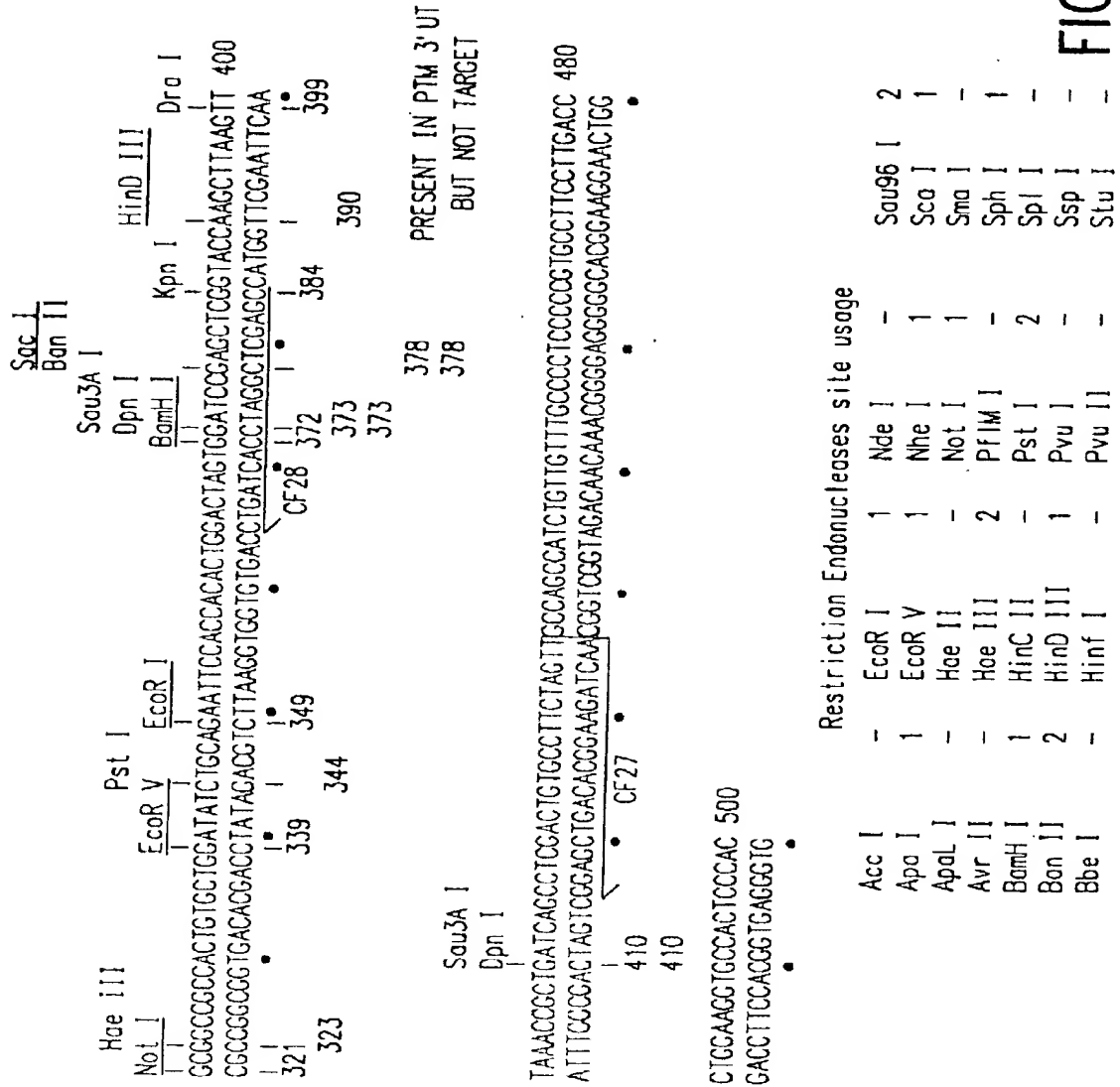


FIG.15A



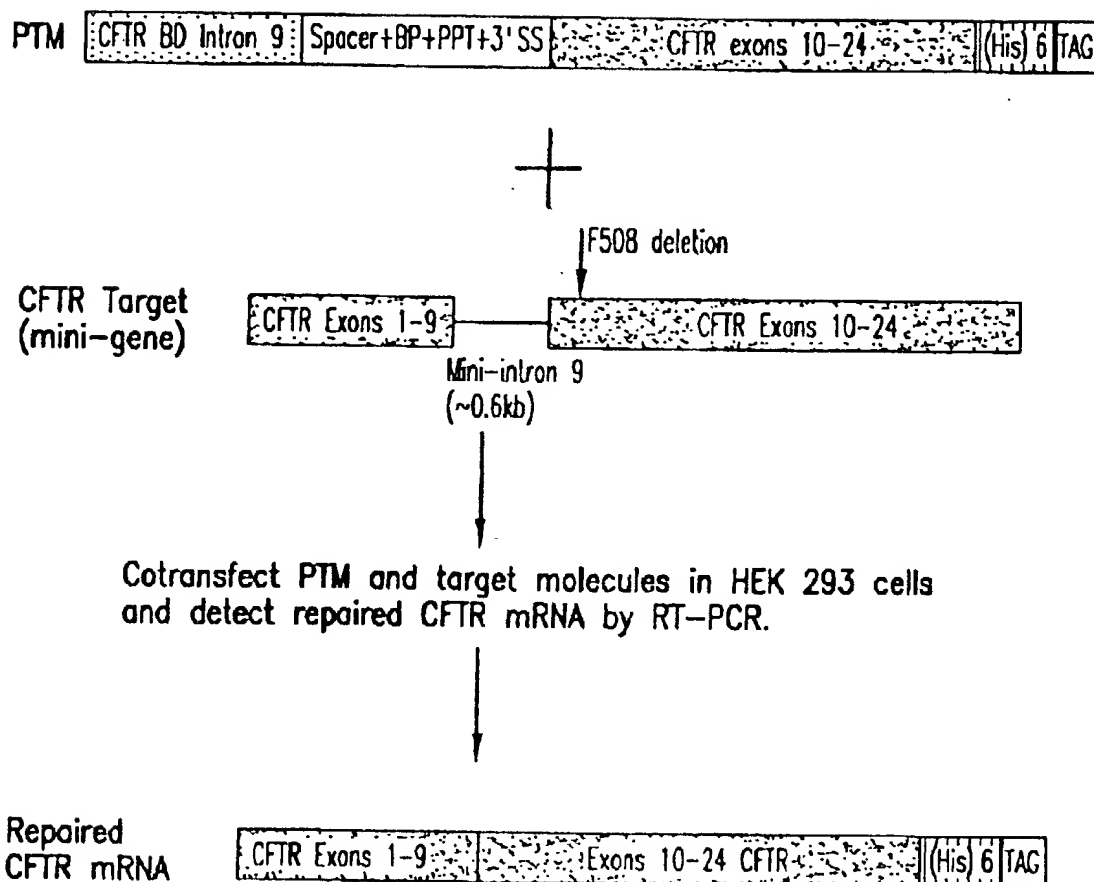


FIG.16

Double Splicing
PTM

CFTR BD intron 9	Spacer+BP+PPT+3'SS	CFTR exon 10	Spacer+BP+PPT+5'SS	CFTR BD intron 10
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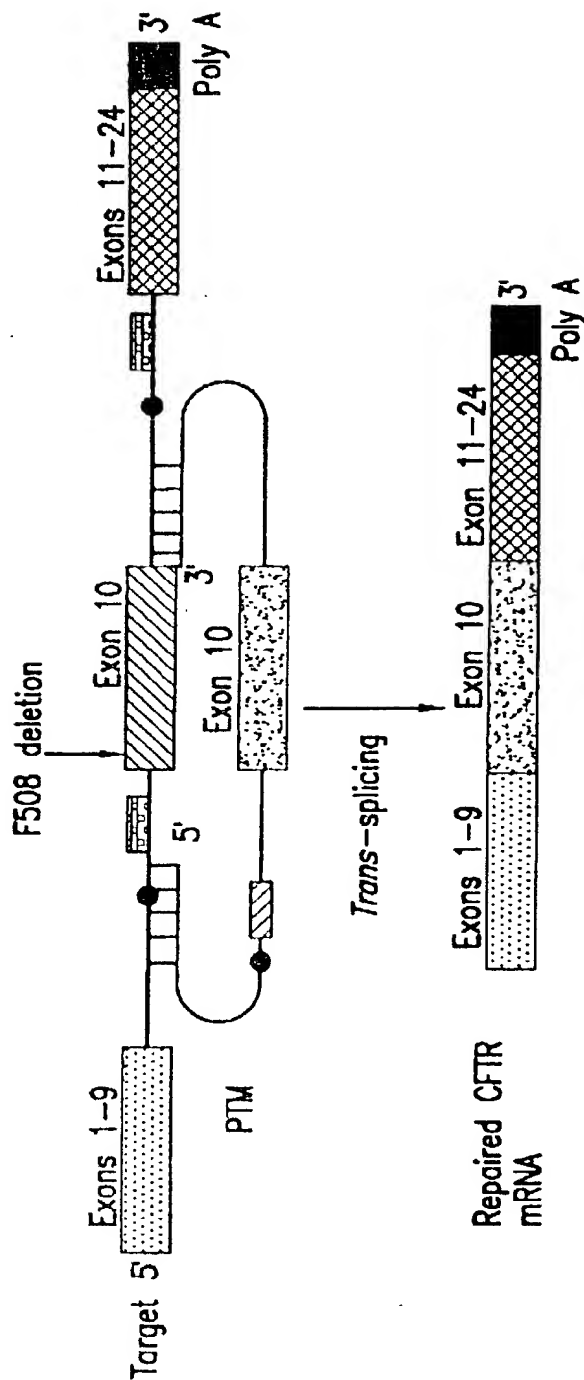


FIG.17

DOUBLE TRANS-SPLICING SPECIFIC TARGET

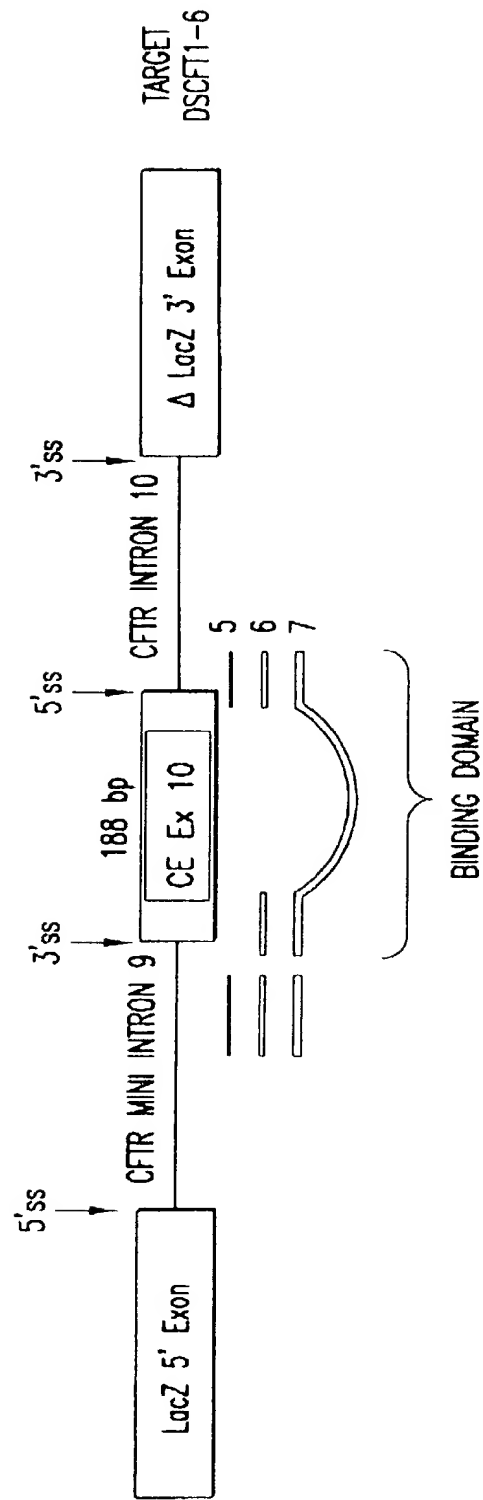


FIG.18

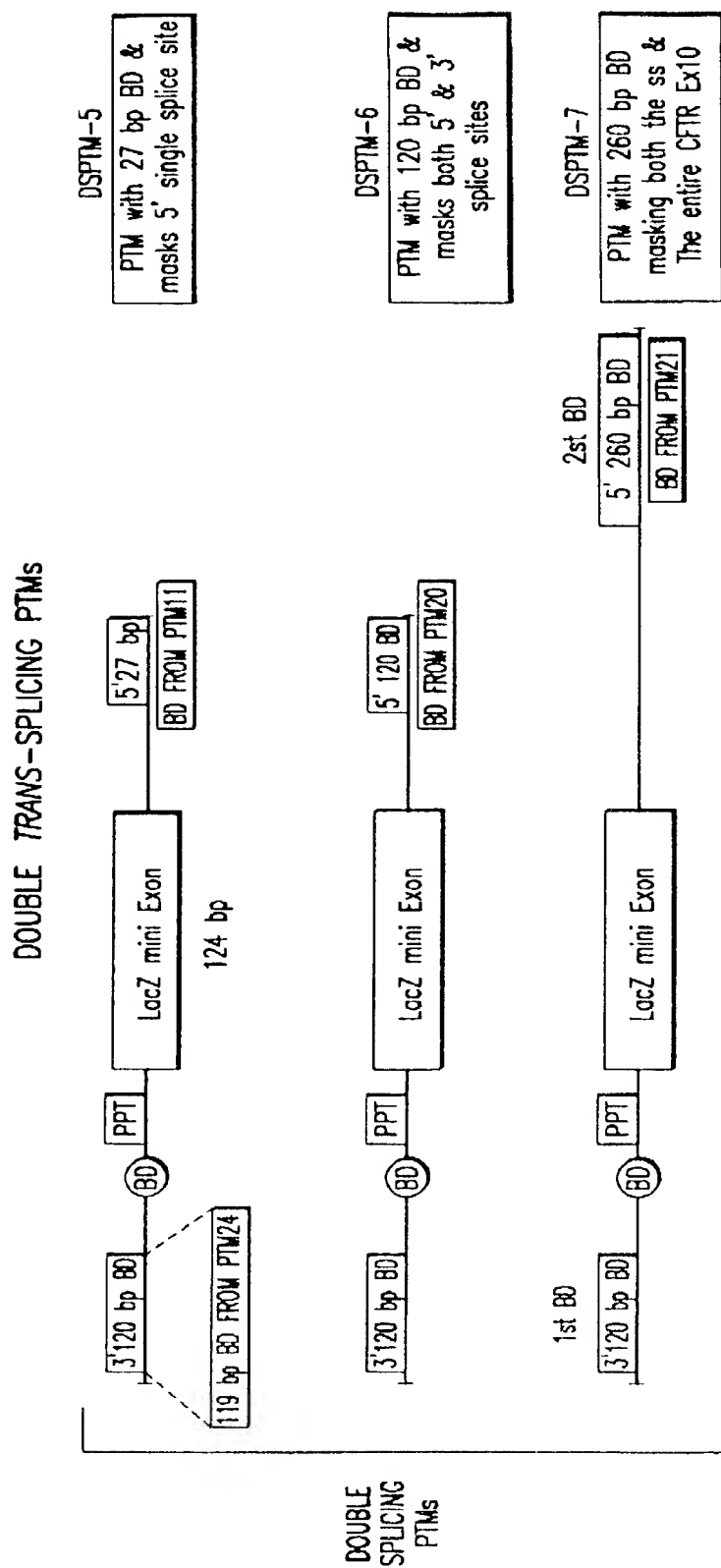


FIG. 19

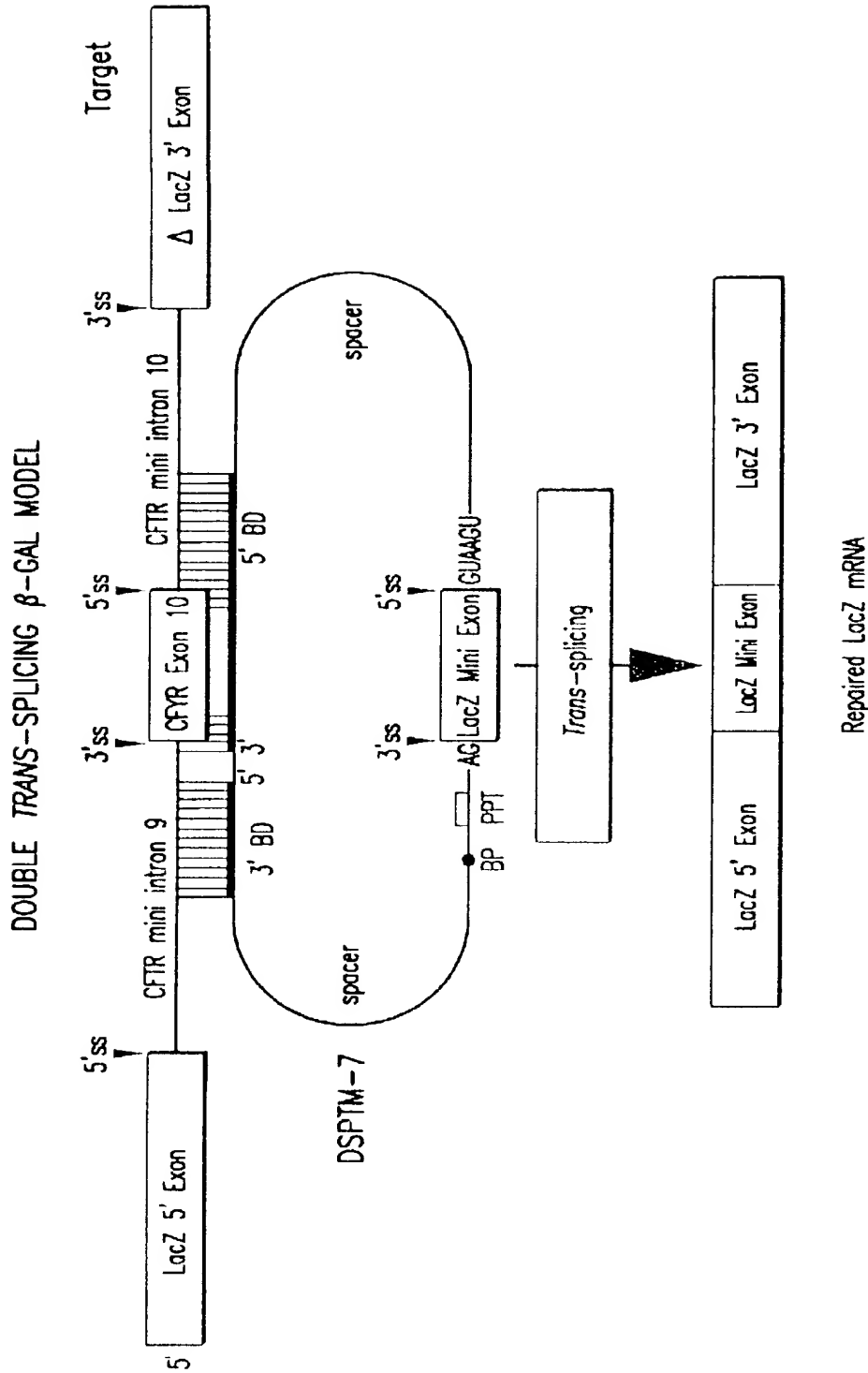


FIG.20

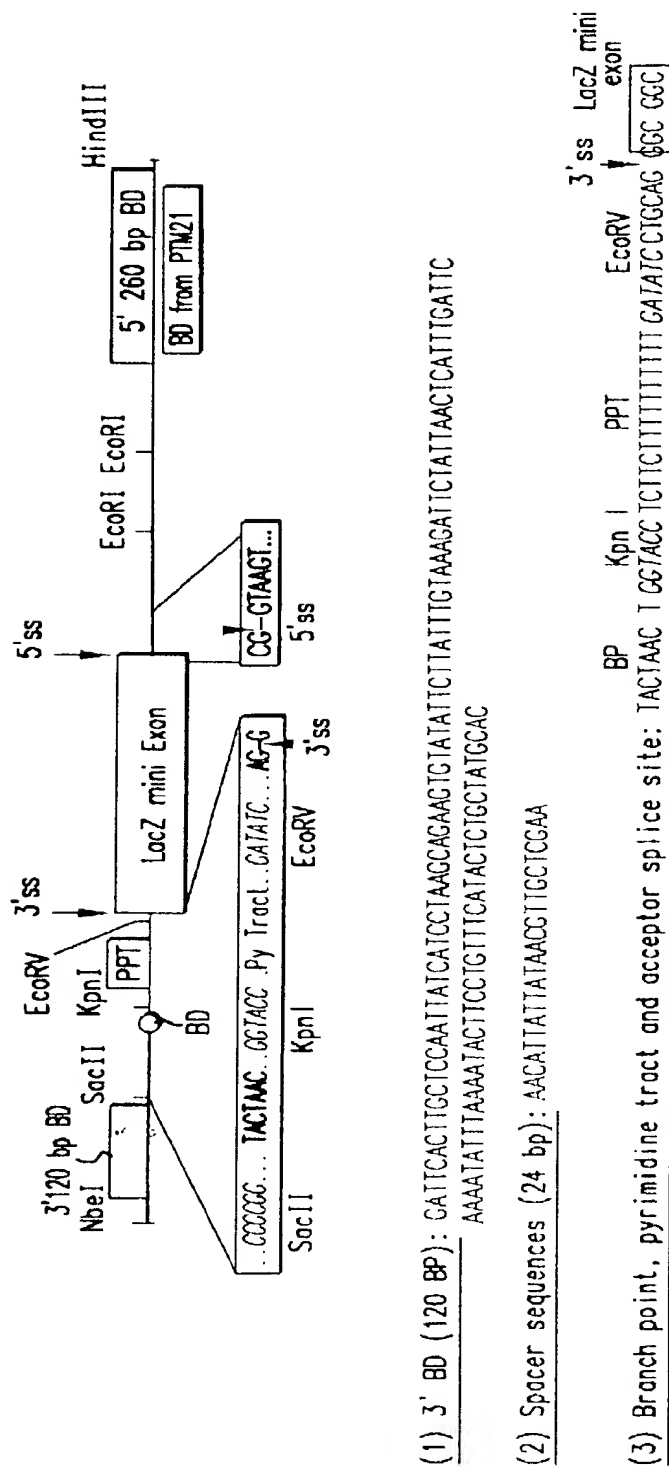


FIG. 21

(1) 3' BD (120 BP): GATTCACITGGCTCCAAITATCAATCCTAAGCAGACGCTATATCTTATTGTGAAGATCTTAACTCATTTCATTC
AAAAATTTAAAAATCTTCGCTGTTTCACTGCTGCTATGCCAC

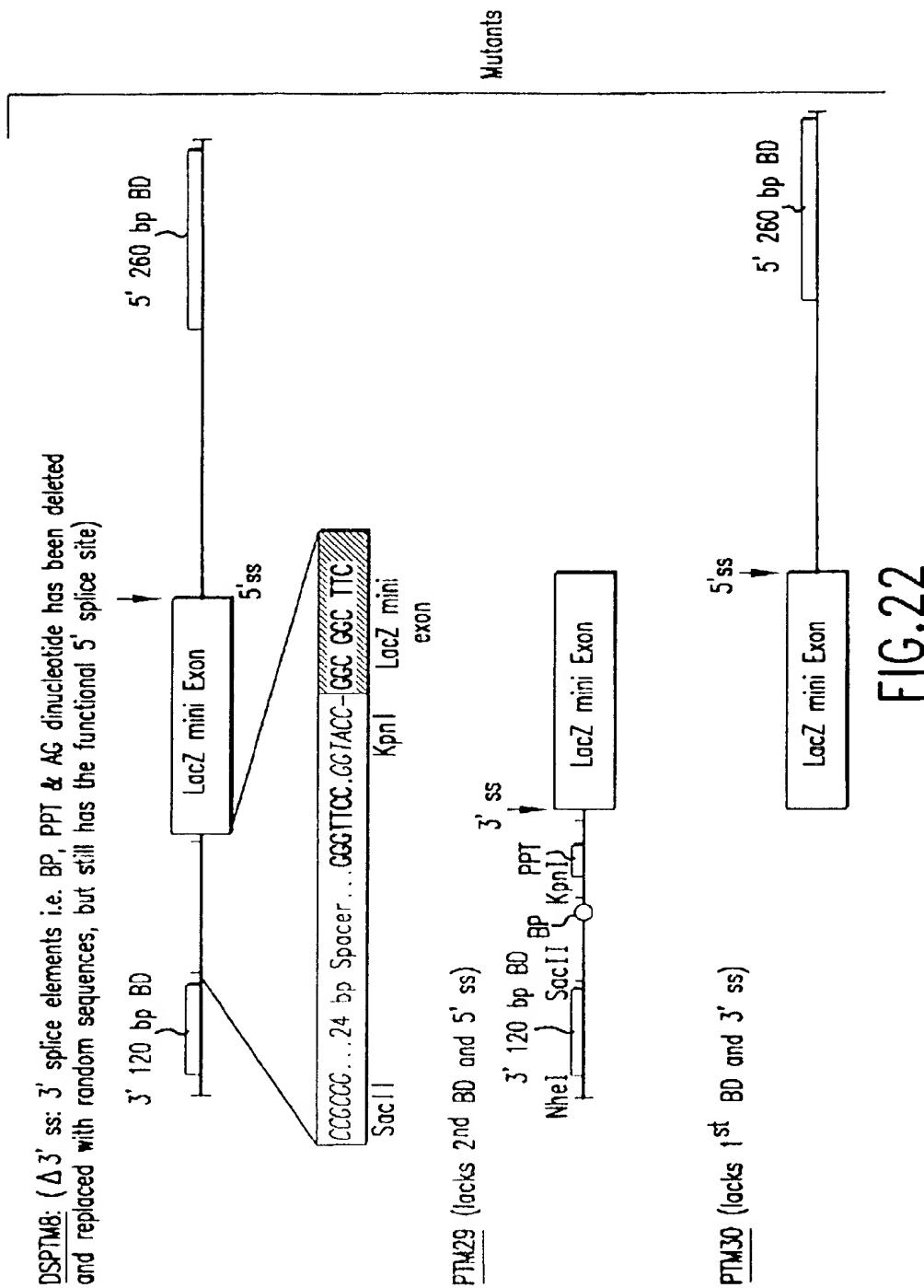
(2) Spacer sequences (24 bp): AACATTATTATAACCTTGCICGAA

(3) Branch point, pyrimidine tract and acceptor splice site: TACTAAC T GGTAAC TCTCTCTTTTTTTT GATATCTGTGCAG GGC GGC

(4) 5' donor site and 2nd spacer sequence: TGA ACG GTAAGT GTTATCACCGATAATGTCGTAAACCTGATTCGGCCCTTCGATACG

CTAAGATCCACCGG

(5) 5' BD (260 BP): TCAAAAGCTTTCACATAATTTCTACCTCTCTTGAATTCATCTCTGATGACGCTCTCTGTACTAATCATCATTCGAAACACCAATGATTTCTTTTAATGGTCCCTGGCATAATCTCGAAACTGATAACACAATGAAATCTCTCCACTGCTTAA
AAAACCCCTCGAATCTCCATTTCTCCATAATCATCATTACAACTGAACCTCTGGAAATAAACCCATCATTTAATCACTCATCAATCAATCAAGCCTTATCAAAATCAGC



Double Trans-splicing Produces Full-length Protein

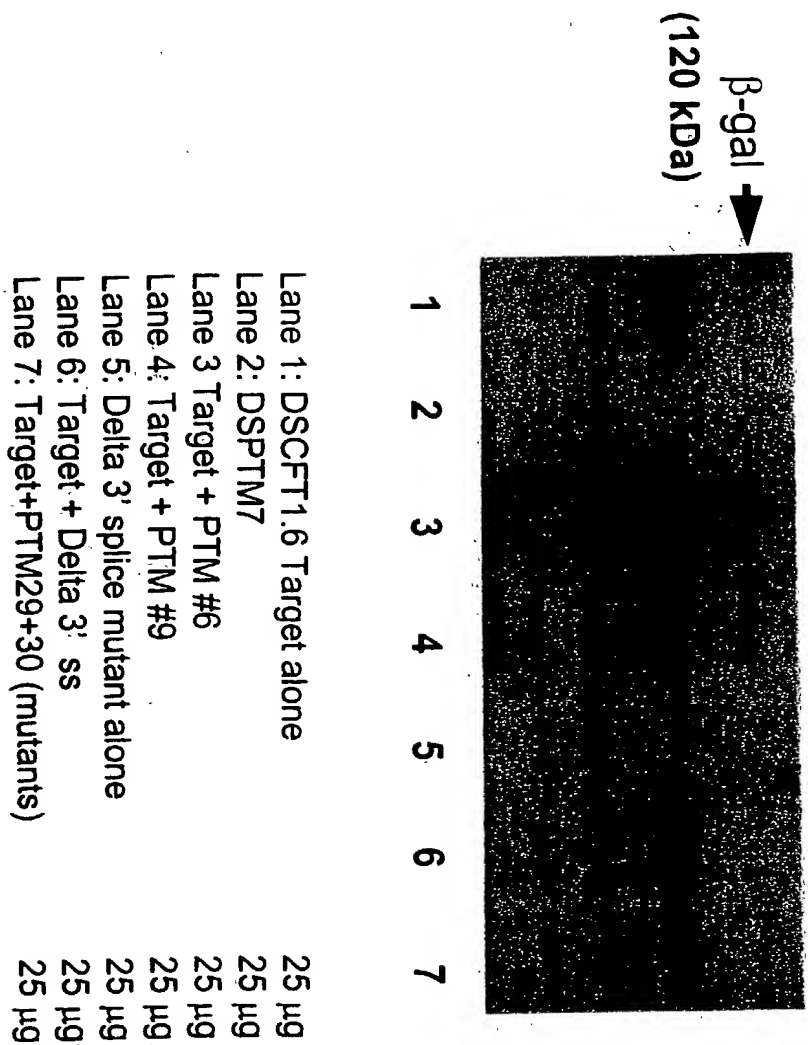


Figure 24

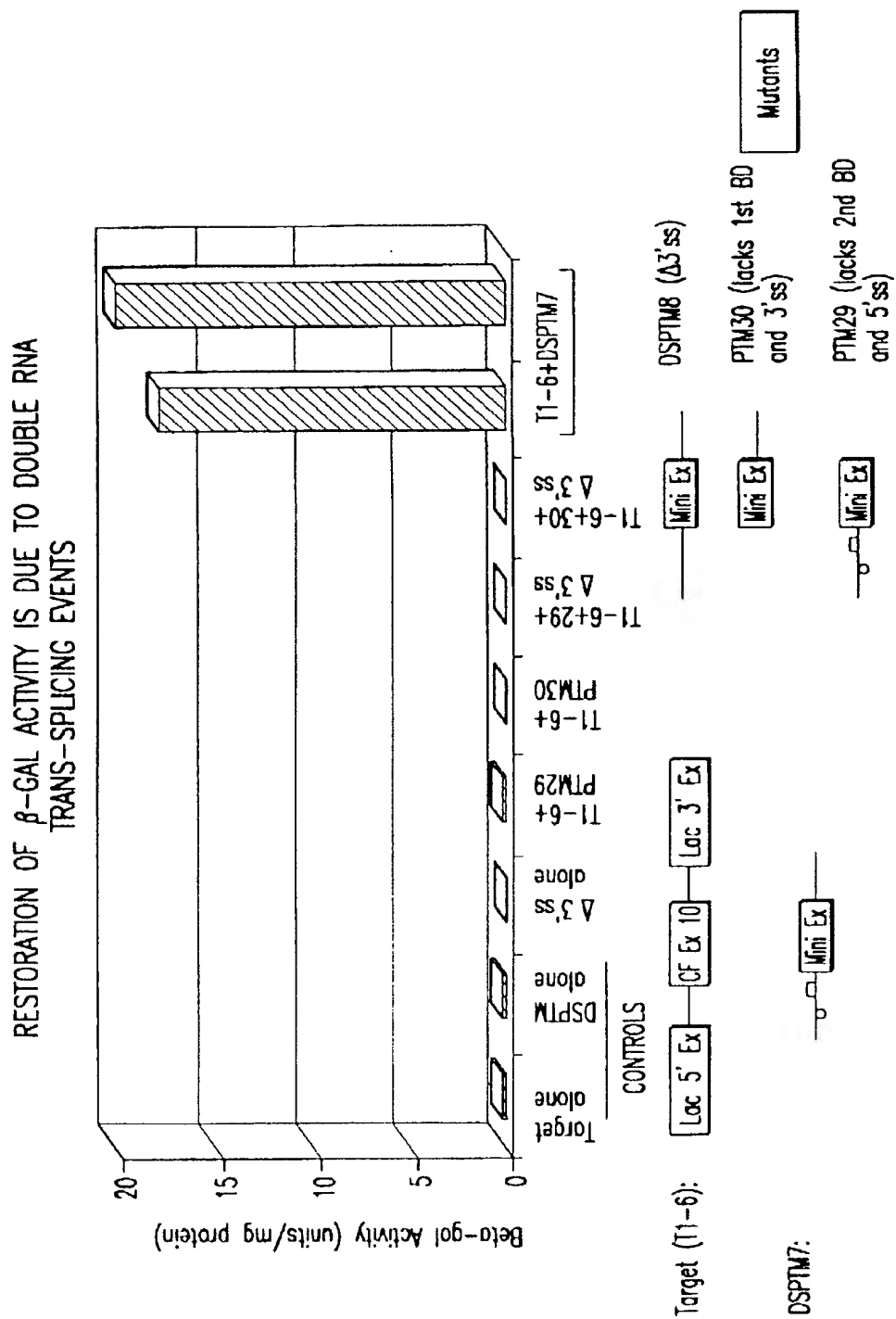


FIG. 26

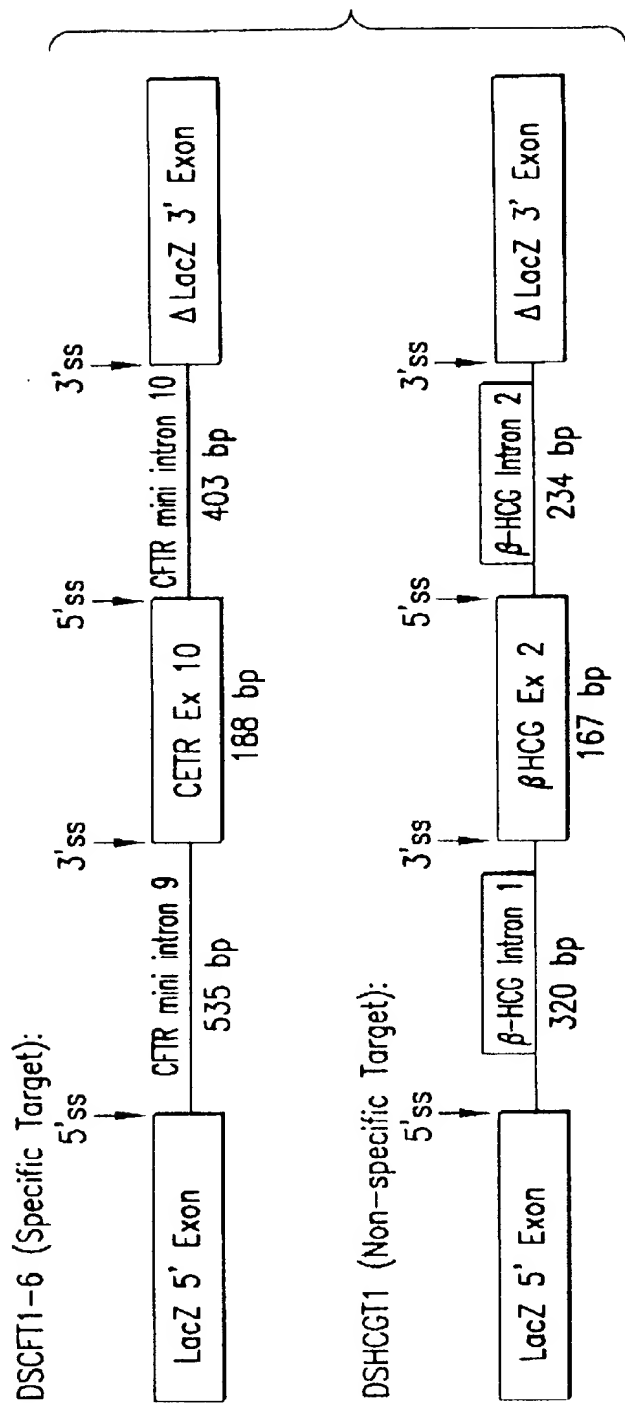
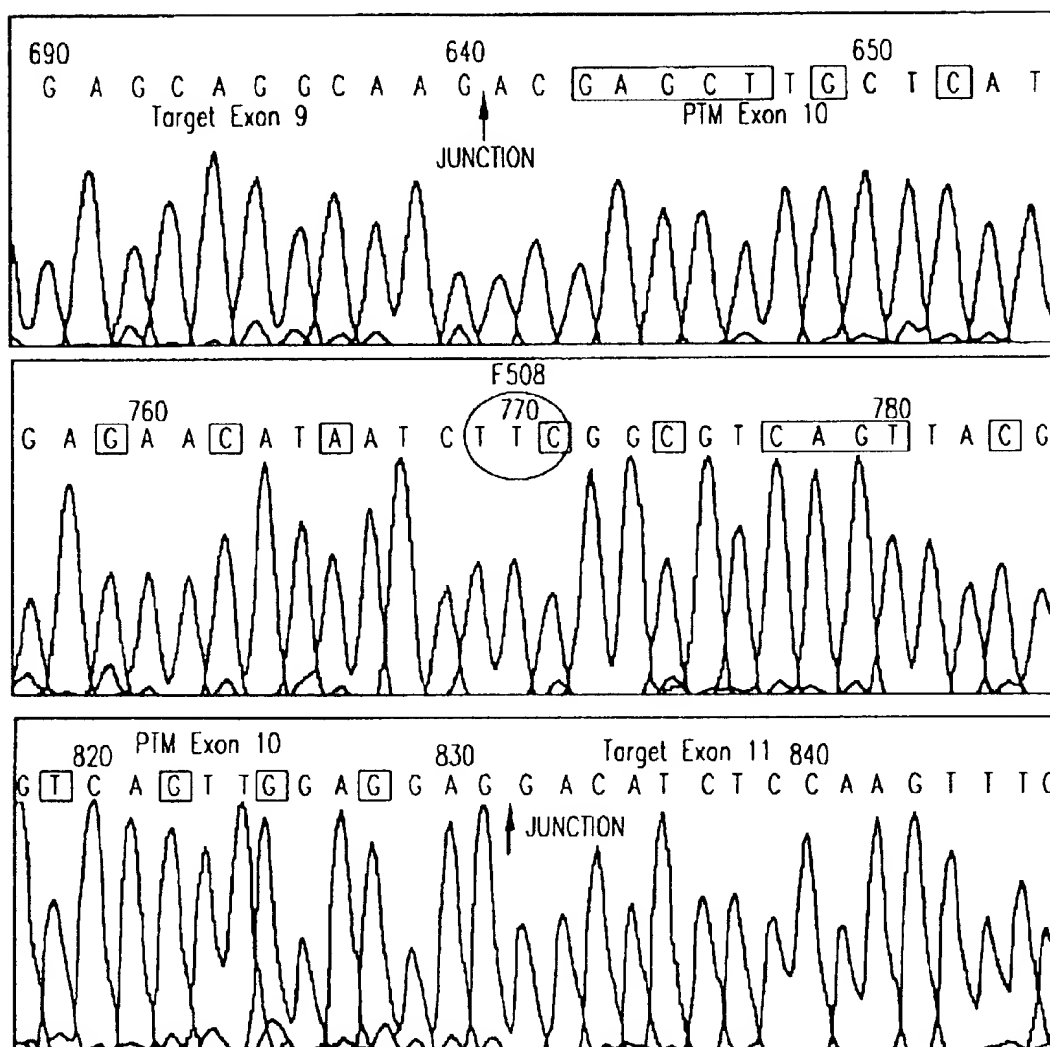


FIG.28

Sequence of a double
Trans-spliced product



□ =MCU in
PTM exon 10

FIG.32

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

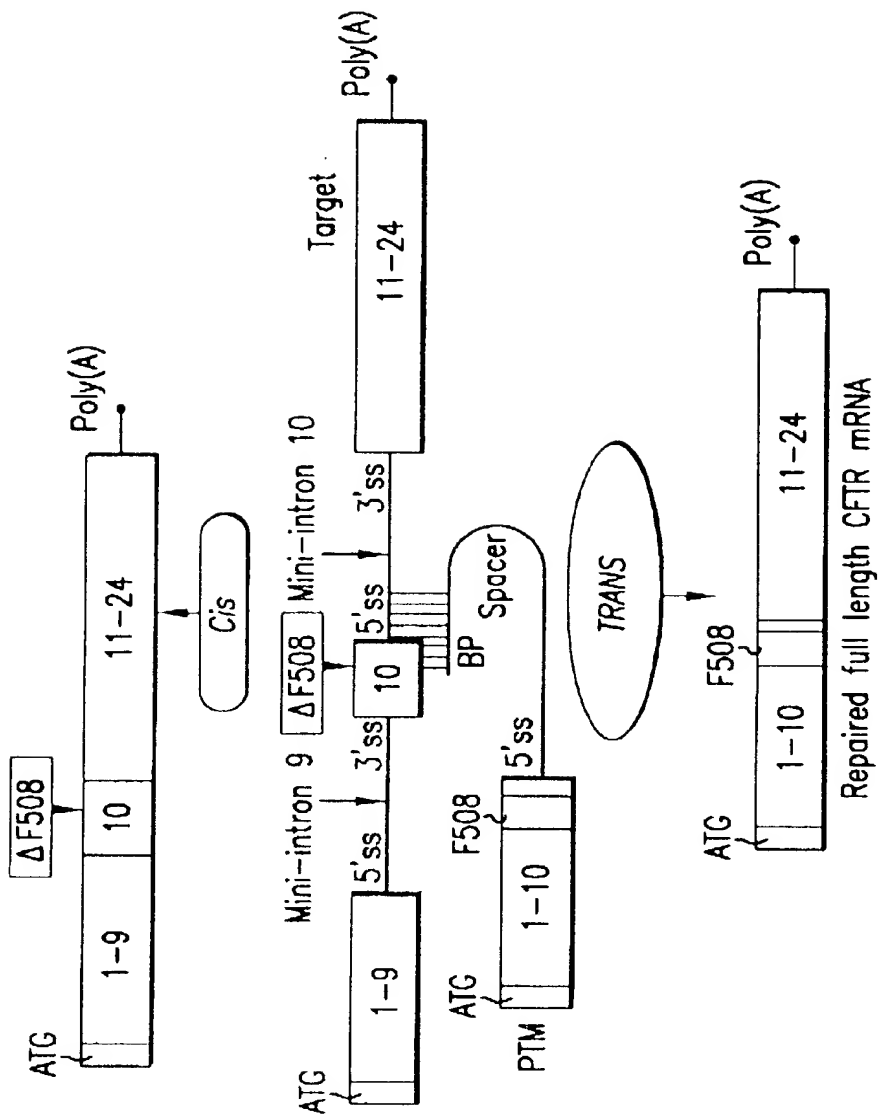


FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target. Target E

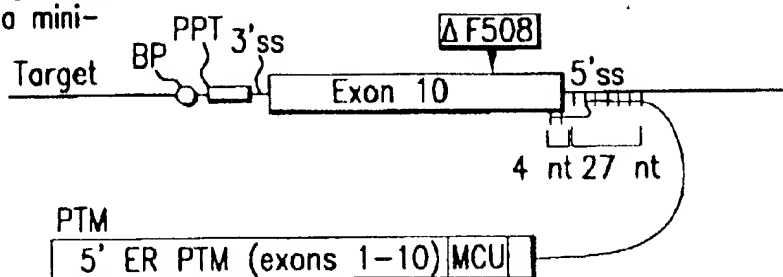


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target. Target

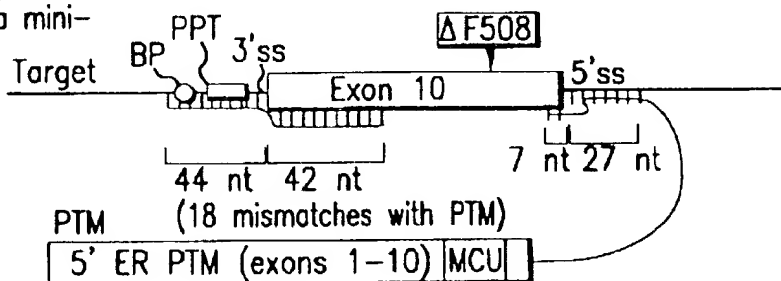


FIG. 34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

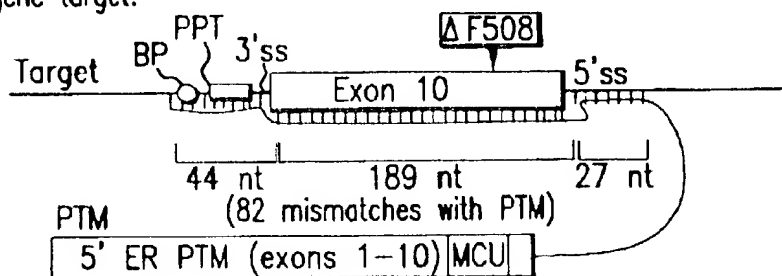
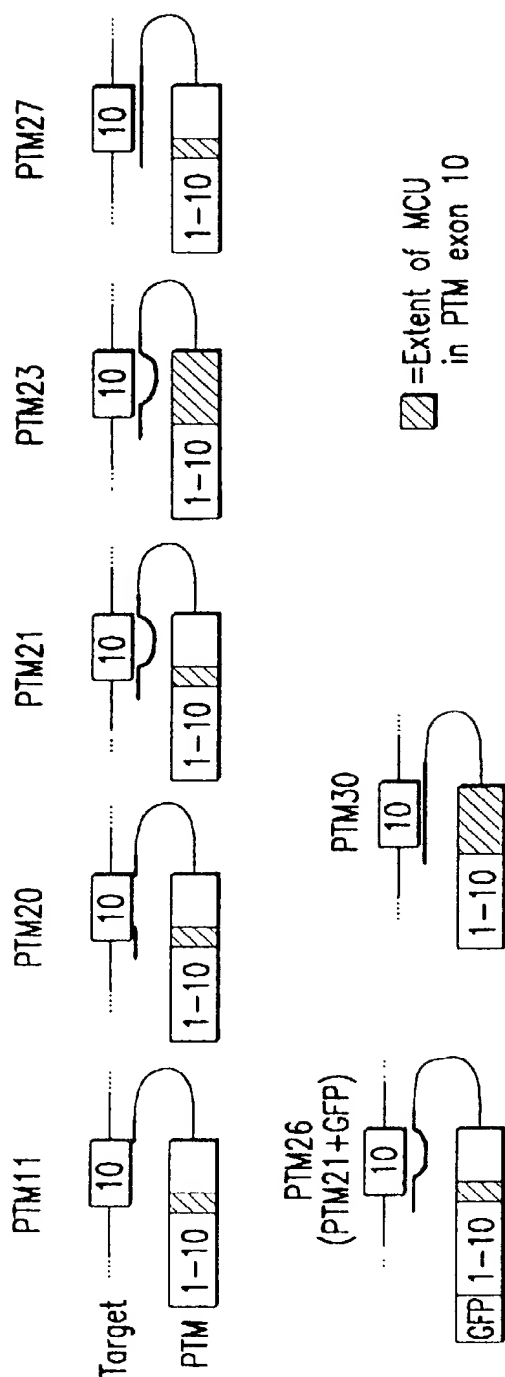


FIG. 34C



MCU in exon 10 of PTM
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGC TCA TGA TGA TCA TGGCGAGTTAGAACCAAGTGAAGCAAGA TCAACATTCGG
GCGGCA TCAGC TTTCCAGCCAA TTCAGTTGGATCATGCCCGGTACCA TCAAGGAGAACA TAA T
CTCGGGCG TCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAAGGCCGTGTCAGTTGGAGGAG

FIG. 35

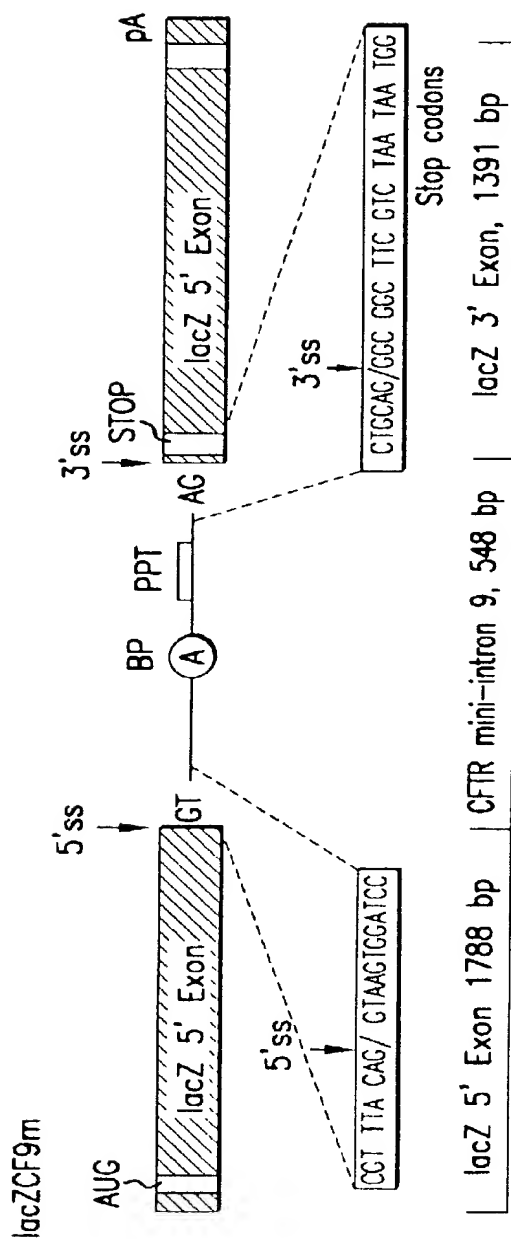


FIG.37A

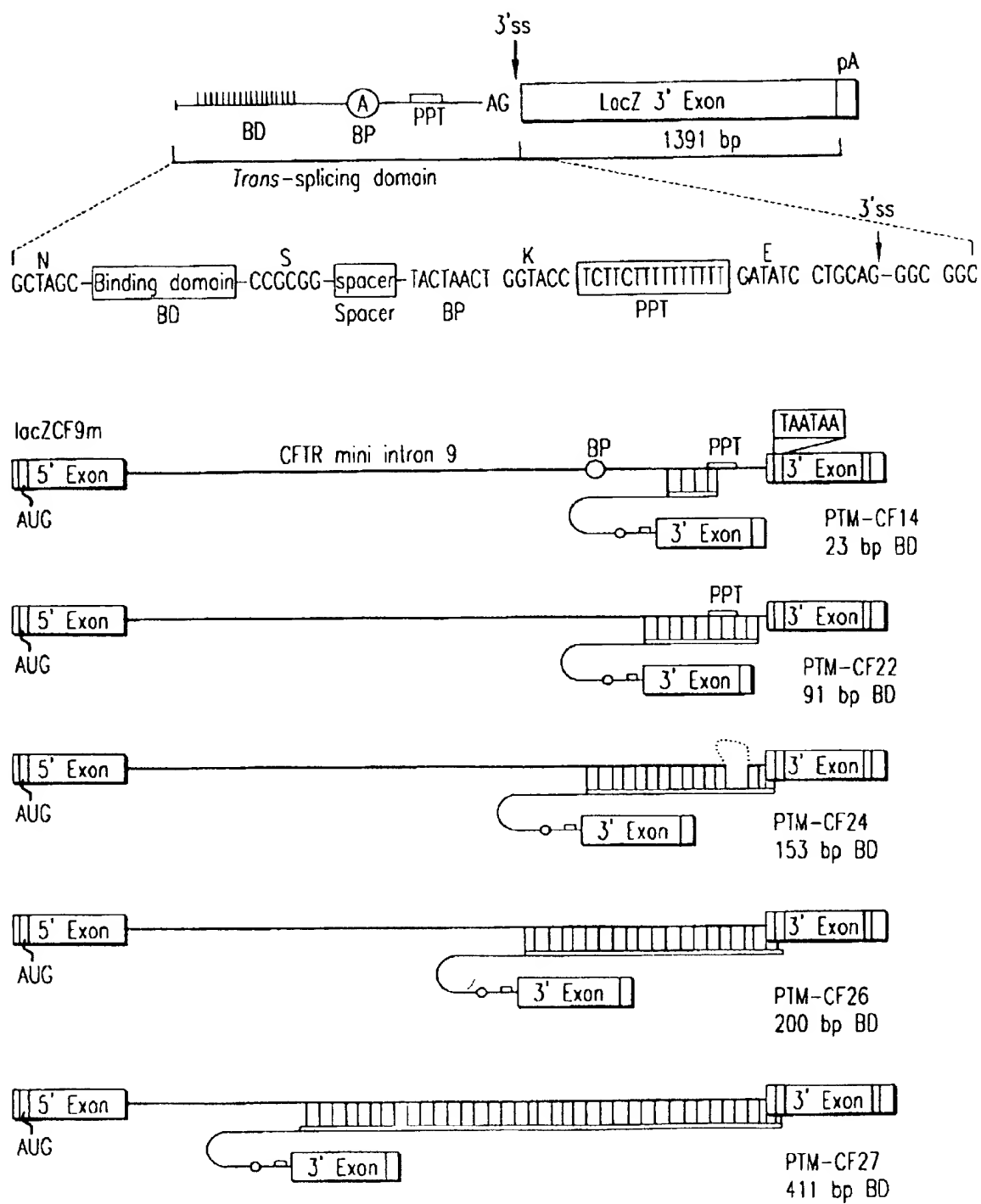


FIG.37B

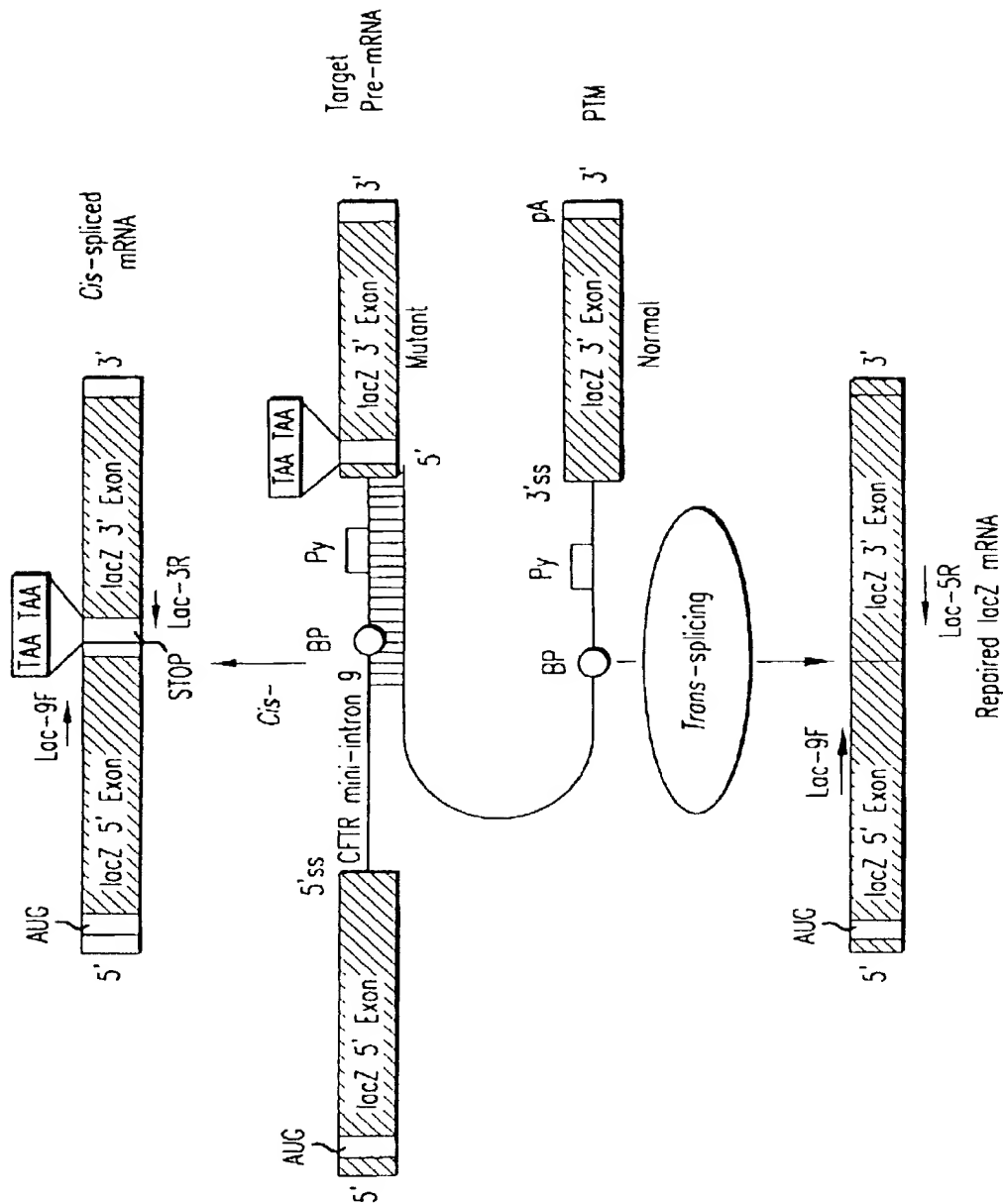


FIG.37C

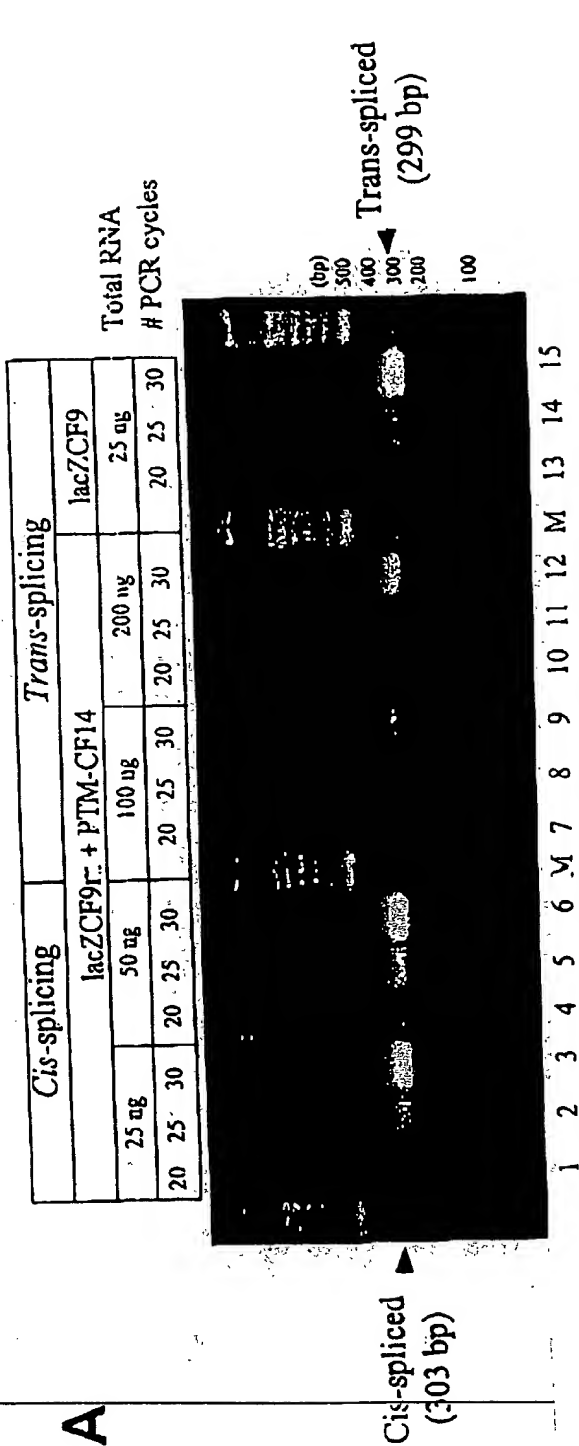
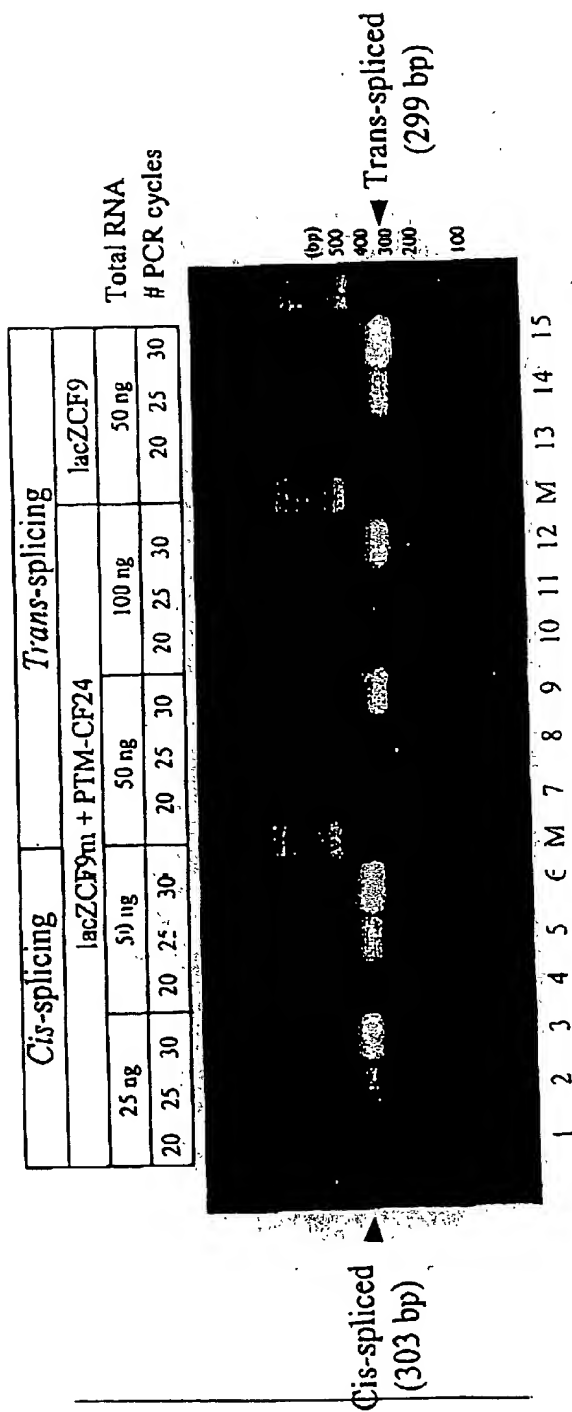
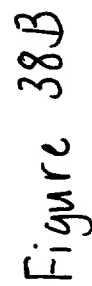


Figure 35A





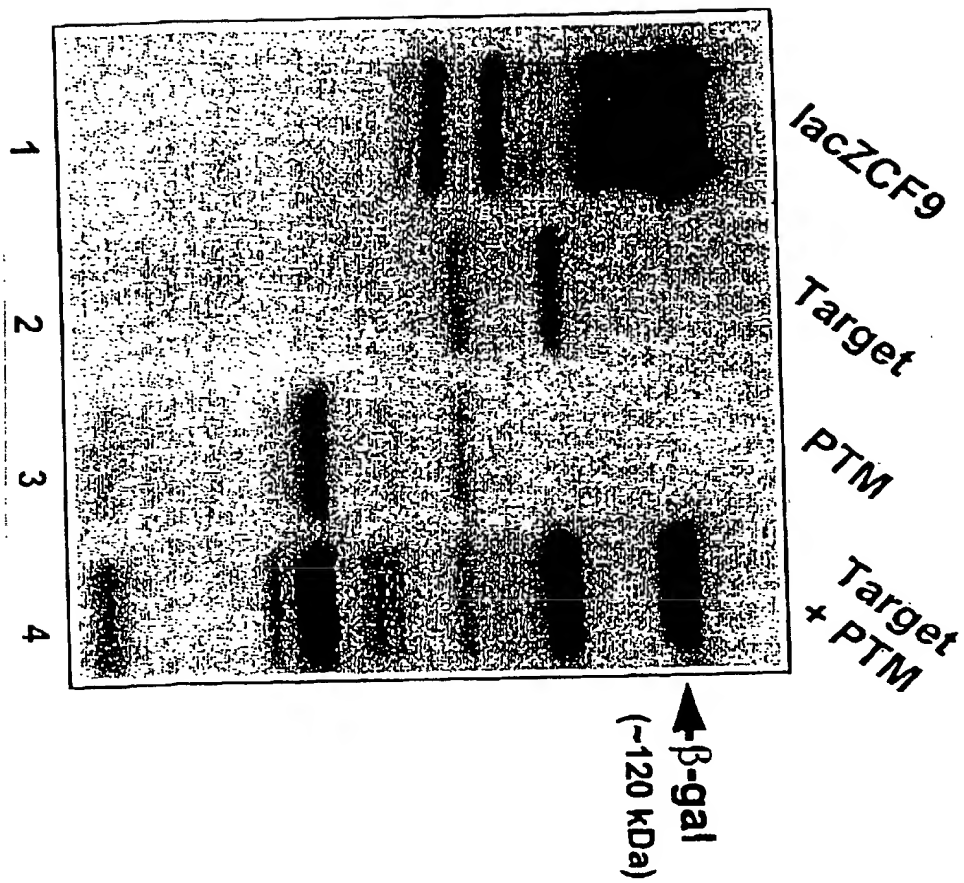


Figure 39

A

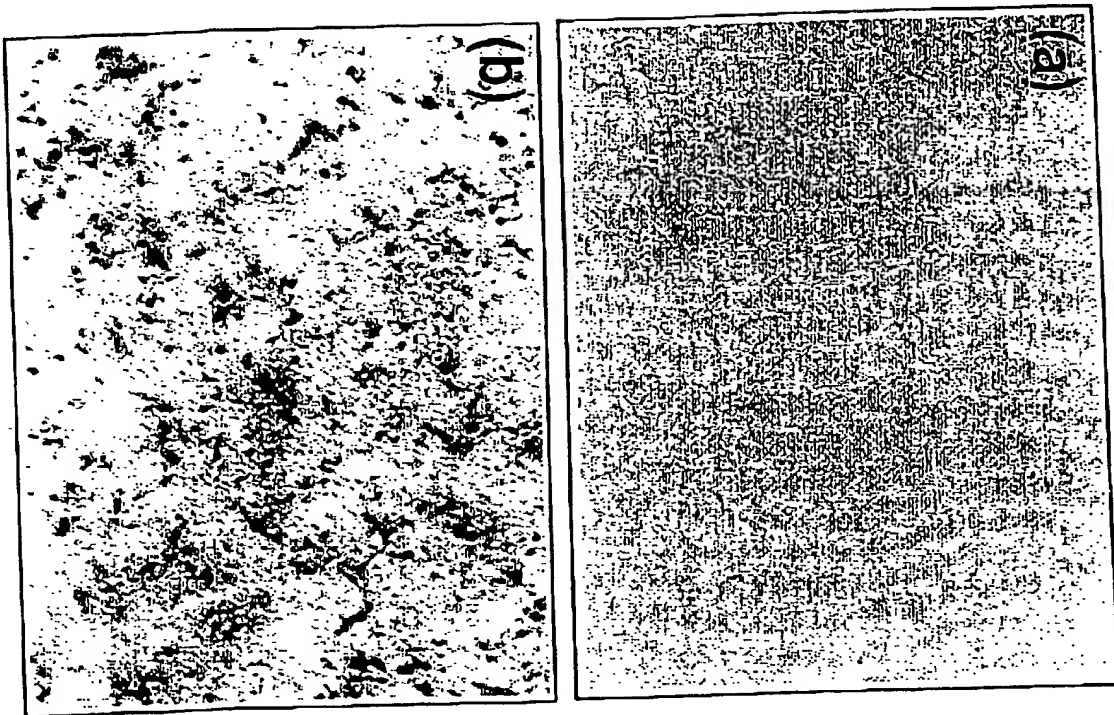


Figure 40A

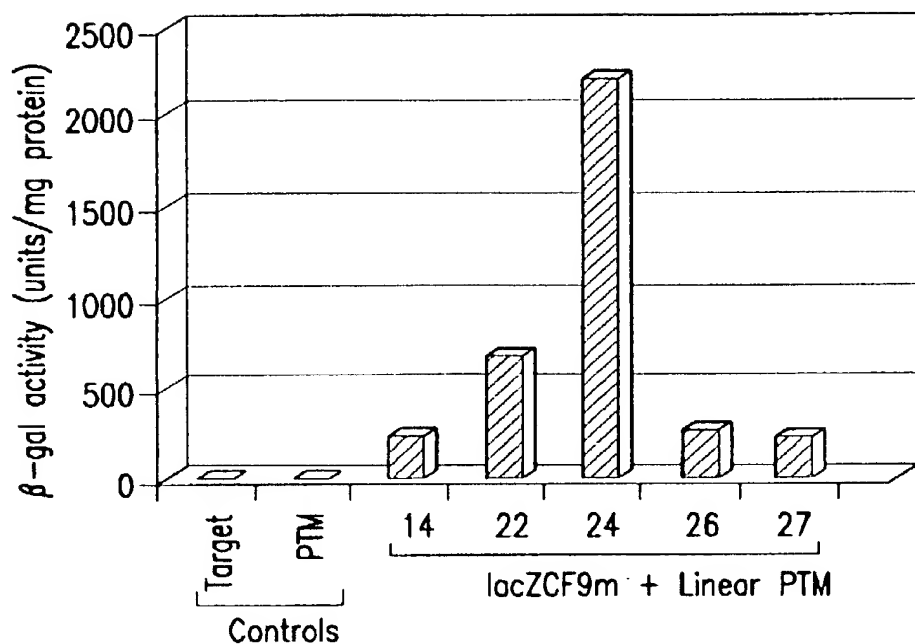


FIG. 40B

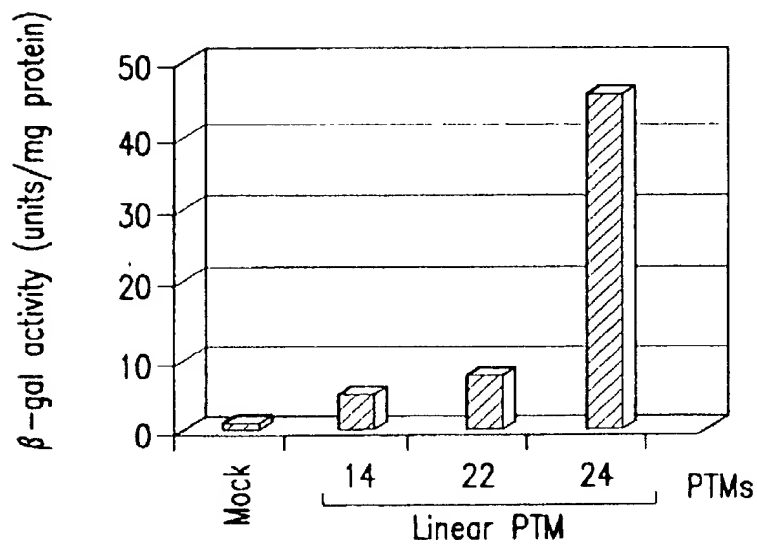


FIG. 40C

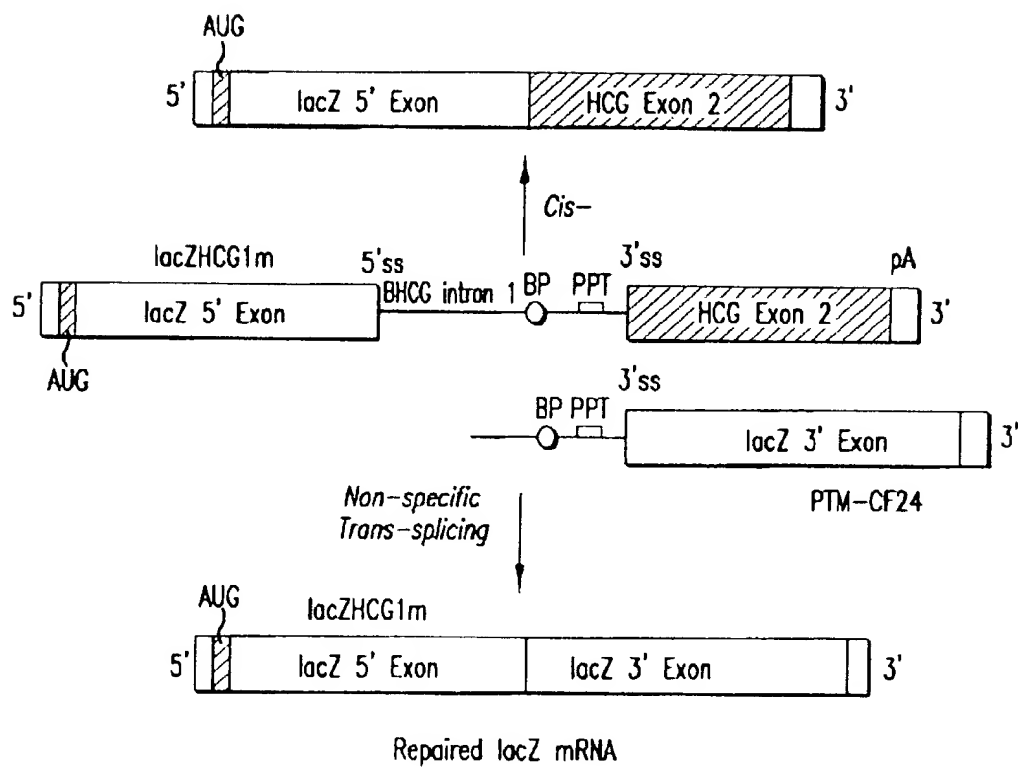


FIG.41A

EXED. # 14

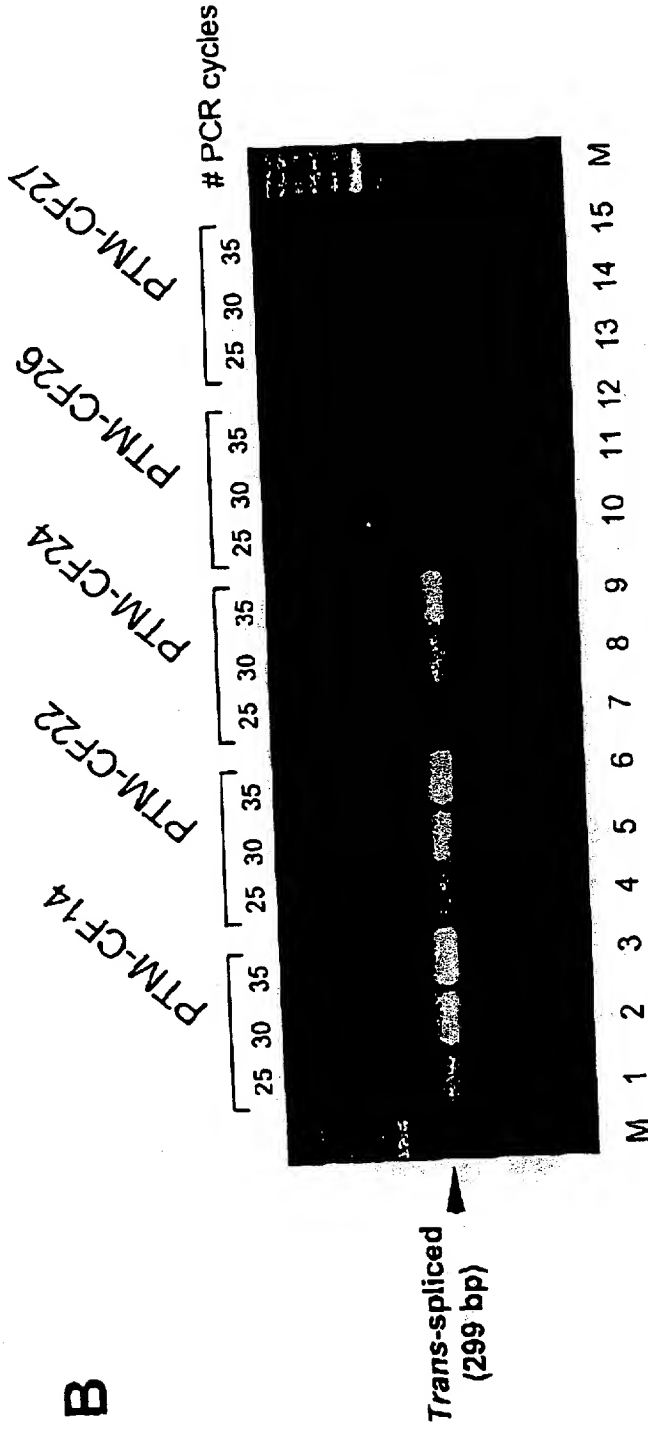


Figure 4CB

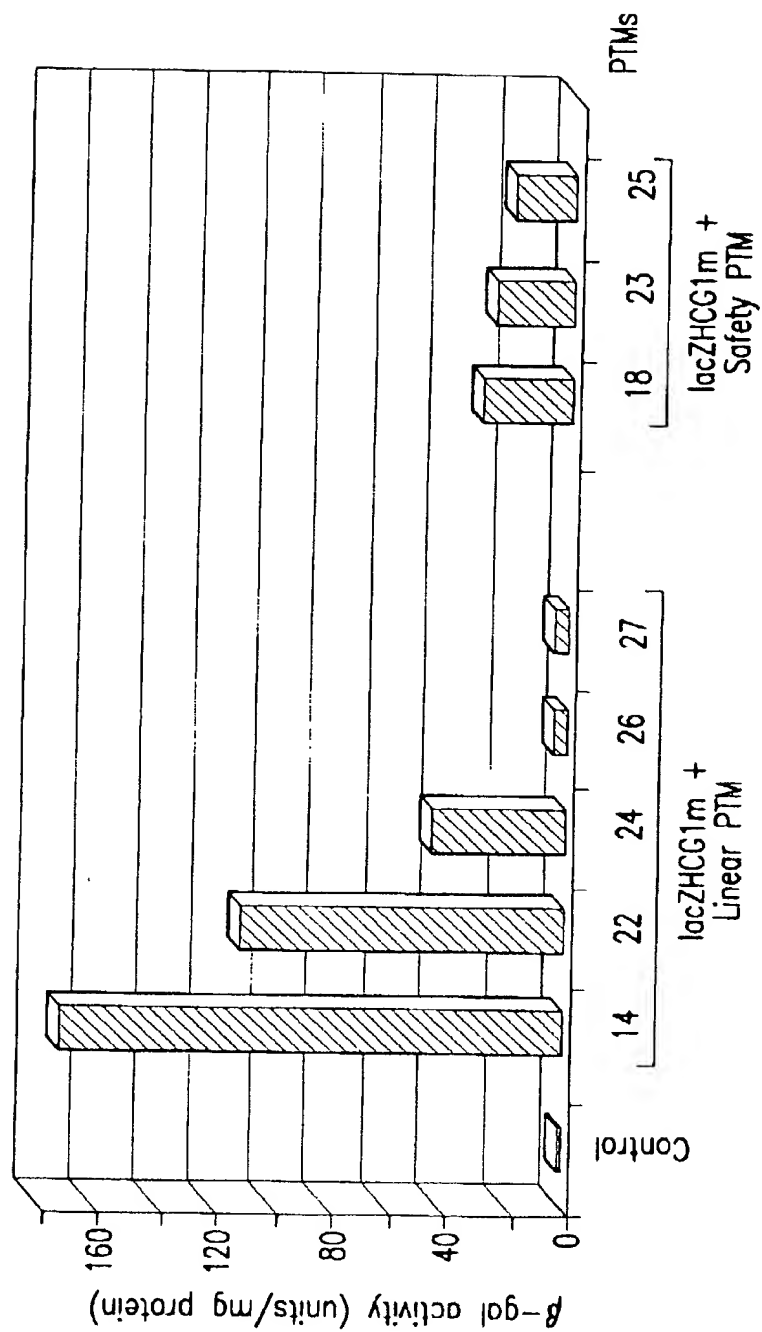


FIG. 41C

Exons

1-10

ATGCAGAGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
GATACAGACAGCGCCTGGAATTGTCAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
GGAAAGAGAATGGGATAGAGAGCTCGCTTCAAAGAAAAATCCTAAACTCATTAAATGCCCTTCGGCGATGTTTTTCTGG
AGATTTATGTTCTATGGAATCTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAACAATCA
TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTAT
TGTGAGGACACTGCTCCTACACCCAGCCATTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT
TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACCTGTTAGTCTCCTTT
CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCACATTTCTGTGGATCGCTCCTTTGCAAGTGGCACTCCT
CATGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCTGATAGTCTTGCCTTTTTCAG
GCTGGGCTAGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAGACTTGTGATTACCTCAG
AAATGATCGAGAACATCCAATCTGTTAAGGCATAGTCTGGGAAGAAGCAATGGAAAAATGATTGAAAACCTTAAGACA
AACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTCTCAGGGTTCTTT
GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGAAAAATATTACCACCATCTCATTCT
GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTGGAGCAATAAA
CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG
AATGTAACAGCCTTCTGGGAGGAGGATTTGGGGAATTATTGAGAAAGCAAAACAAACAATAACAATAGAAAACTT
CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCAGTTCTTGGTACTCCTGTCTGAAAGATATTAATTTCAAGAT
AGAAAGAGGACAGTTGTTGGCGGTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGCGGAG
TTAGAACCAAGTGAAGCAAGATCAACATTCGGGCGGCATCAGCTTTTGCAGCCAAATCAGTTGGATCATGCCCGGTA
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGAGAGTACCGCTATCCCTCGGTGATTAAAGCCCTGTCAGTTGGA
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTCGGGCCCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTC
GAAACACCAATGATATTTCTTTAATGGTGCCTGGCATAATCCTCGAAAACGATAACACAATGAAATCTTCCACTGT
GCTTAATTTTACCCTCTGAATTCCTCATTTCTCCATAATCATCATTACAACCTGAACTCTGGAAATAAAACCCATCATT
ATTAACCTATTATCAATCAGCT

FIG.42

153 bp PTM24 Binding Domain:

Nhe I

153 bp BD underlined

GCTAGC-AATAATGACGAAGCGCGCCCTCAGGCTCAGGATTCACTTGCCTCCAATTATCATCCTAAGCAGAAGTGATA

TTCCTATTGTAAGATTCTTAACTCATTGATTCAAAATATTTAAATACCTCCTGTTTACCTACTCTGCTATGC

Sac II

AC-CCGCGG

FIG. 43A

FIG. 43B